INFLUENCE OF RHIZOSPHERE MICROORGANISMS ON THE GROWTH OF *UNIOLA PANICULATA*

By

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Plants must be quickly established on renourished beaches in order to stabilize the sand and begin the dune-building process. Experiments were conducted to determine whether inoculation of sea oats with bacteria (indigenous rhizosphere bacteria and N_2-fixers) and vesicular arbuscular mycorrhizal (VAM) fungi would enhance early plant growth in sand under greenhouse conditions. At two fertilizer-N levels, Klebsiella pneumoniae and two Azospirillum spp. did not provide the plants with fixed atmospheric N; however, these bacteria had a positive effect on root and shoot growth. Bacterial inoculation did not increase the plant uptake of water-soluble P in native sand, but when soluble-P was added there were increases in plant growth and shoot P resulting from bacterial inoculation. When a sparingly-soluble P source was added to two sands, increases in growth and shoot P due to bacterial inoculation varied with sand source. When sea oats, growing in two sands, were dually-inoculated with either K. pneumoniae or A.

denitrificans and a combined *G. deserticola*-*G. macrocarpum* VAM fungi inoculum there was no consistent evidence of a synergistic effect on plant growth, although plant response to inoculation varied with sand source. Bacterial inoculum had no effect on VAM fungi colonization of roots when the inoculum contained spores alone but did when root-hyphae-spore inoculum was used. *Klebsiella pneumoniae* appeared to induce early spore germination and faster hyphal growth; however, this did not translate into earlier or more successful root colonization by VAM fungi. The effects of inoculation of sea oat seedlings with bacteria and VAM fungi in a commercial potting medium were explored. Inoculation with VAM fungi increased plant growth whereas *K. pneumoniae* had a negative effect on growth. However, *K. pneumoniae* stimulated VAM fungi root colonization in Metro Mix, whereas the VAM fungi alone were slow to colonize the roots of seedlings. Further research is needed to match bacterial and fungal coinoculants with growing media and fertilizer regimes.

CHAPTER 1
INTRODUCTION

Accelerated coastal erosion threatens private and public property in many areas of the world. Erosion in Florida is considered critical by its residents because of continuing high investments in shoreline development and the revenues generated by the tourist industry (Callahan, 1980).

Several techniques for the replacement of lost beach sand and its stabilization have been suggested and applied with varying degrees of success (Dean, 1976). A generally accepted procedure is to replenish the beach with material of compatible physical properties dredged from estuarine or offshore locations, or borrowed from inland sites. The material is shaped to the desired beach profile and the back beach may be planted with appropriate pioneer species, such as sea oats (Uniola paniculata L.) and panic grass (Panicum spp.), to enhance beach stability and begin the dune-building process.

The survival of the transplanted grasses depends on many factors, including temperature, moisture and nutritional stresses, and the effects of wind, wave action, and salt spray. The major factors limiting the vigorous establishment and growth of the plants in the face of environmental extremes are the infertility and poor moisture holding capacity of coarse dune-nourishment materials (Kachi and Hirose, 1983;

Woodhouse, 1982; Atkinson, 1973; Willis and Yemm, 1961). Nitrogen (N) was the limiting nutrient in field studies with Ammophila arenaria (Willis, 1965) and Oenothera erythrosepala (Kachi and Hirose, 1983). The work of the latter author suggested that restricted N mineralization and nitrification, and leaching of nitrate-N resulted in low-N contents in alkaline sand dunes. Skiba and Wainwright (1984) also found nitrification was inhibited and nitrate leached rapidly in immature calcareous dune sands. Toxic nitrite accumulations were evident in highly alkaline sand.

Under many circumstances, fertilization of young pioneer plants beyond an initial treatment is not economically feasible. Various natural sources of plant nutrients such as salt spray (van der Valk, 1974; Wilson, 1959), freshly deposited sand (Wagner, 1964), and rainfall (van der Valk, 1974) may contribute to plant growth in coastal dunes. Although small amounts of nitrate and cations are contributed to plants from these sources, rapid leaching of the sand precludes the accumulation of nutrients in the rhizosphere.

It has been suggested that rhizosphere microorganisms may enhance dune plant growth (Sylvia and Burks, 1988; Koske and Polson, 1984; Abdel Wahab and Wareing, 1980). Scanning electron microscope studies of the distribution of microorganisms on, and in, roots of dune grass have indicated possible nutritional roles for bacteria and fungi (Old and Nicolson, 1975; Marchant, 1970). Asymbiotic N_2-fixing bacteria have been shown to increase plant N (Abdel Wahab and Wareing, 1980) and plant growth (Smith et al., 1976). The role of mycorrhizal fungi in increasing growth and P content of dune grasses has been documented

(Sylvia and Burks, 1988; Nicolson and Johnston, 1979). Therefore, a series of greenhouse experiments were conducted as part of a Sea Grant-funded project to evaluate the role of rhizosphere microorganisms in the survival and growth of sea oats on beach dunes in Florida. The following chapters document these experiments and compare the results to the extant literature.

In Chapter 2, the hypothesis tested was that inoculation of sea oat seedlings with selected bacteria would enhance their growth and shoot N status. The objectives were to (1) measure the N_2-fixing ability of various bacteria, including several isolates indigenous to sea oat rhizospheres and others known to fix atmospheric N_2; (2) select bacterial isolates for use as inoculum in greenhouse experiments; (3) compare root and shoot growth, and shoot N and P content of sea oats grown in sand at two N levels, with and without bacterial inoculation; and (4) evaluate the ability of the N_2-fixing bacteria to provide greenhouse-grown sea oat plants with adequate N for growth, as compared to that achieved with N fertilization.

In Chapter 3, the hypothesis tested was that inoculation of sea oat seedling with selected bacteria would enhance P uptake by plants. The objectives were to (1) compare growth, and root and shoot N and P content of sea oats grown in sand at two soluble fertilizer-P levels, with and without bacterial root inoculation; (2) evaluate the ability of selected bacterial isolates to solubilize sparingly-soluble calcium phosphate; (3) compare growth, and shoot N and P content of sea oats grown in two sands that differed in N and P content, and were amended

with sparingly soluble calcium phosphate, with and without bacterial root inoculation.

In Chapter 4, the hypothesis tested was that dual inoculation of sea oat seedlings with vesicular-arbuscular mycorrhizal (VAM) fungi and a selected bacterial isolate would enhance the growth and nutrient status of plants grown in sand. The objectives were to (1) evaluate the ability of two bacterial isolates to increase root colonization by the VAM fungus inoculum that consisted of a mixture of Glomus deserticola Trappe, Bloss and Menge (isolate 305) and Glomus macrocarpum Tul. and Tul. (isolate S328), from spore inoculum, and to increase plant growth and shoot nutrient content; (2) evaluate the ability of inoculation with Klebsiella pneumoniae strain Beijing, and the VAM fungi to increase plant and nutrient status of sea oats grown in sand at two P levels; (3) compare growth and nutrient status of sea oats grown at two P levels in two sands and inoculated, or not, with a combination of two bacterial isolates and the G. deserticola-G. macrocarpum mixture from root-hyphae-spore inoculum; (4) to evaluate the effect of a bacterial co-inoculant on colonization of sea oat roots by the VAM fungi.

In Chapter 5, the hypothesis tested was that K. pneumoniae produced a volatile substance which affected spore germination and early hyphal extension of G. deserticola and G. macrocarpum. The objectives were to (1) compare the percentage of spores germinated and germ tube lengths in the presence and absence of K. pneumoniae, in vitro; (2) determine if the presence of K. pneumoniae on filter-paper disks buried in sand and containing the VAM fungal spores influenced spore germination, sea oat root growth, and VAM fungal colonization through time.

In Chapter 6, the hypothesis tested was that inoculation of roots of sea oat seedlings with bacteria and VAM fungi would enhance the plant's ability to adapt to the dune environment when outplanted from a commercial nursery. The objectives were to (1) determine the effect of bacterial root inoculation on shoot growth and nutrient content, and growth of roots from the potting media into sand; (2) determine the effect of dual inoculation with bacteria and VAM fungi on shoot growth and nutrient content, and growth of roots from the potting media into sand; (3) assess root colonization by a G. deserticola-G. macrocarpum mixture.

In Chapter 7, results of the research are summarized and discussed, and conclusions are presented.

CHAPTER 2
GROWTH ENHANCEMENT OF *UNIOLA PANICULATA* RESULTING FROM BACTERIAL INOCULATION AND NITROGEN FERTILIZATION

Introduction

The association between N_2-fixing bacteria and grasses is well established (Patriquin et al., 1983; Brown, 1982; van Berkum and Bohlool, 1980; Neyra and Dobereiner, 1977; Dobereiner and Day, 1976; Nelson et al., 1976). Potential beneficial effects of these organisms on plant growth may result from bacterial N fixation, production of plant-growth-promoting substances, or other factors. Abdel Wahab and Wareing (1980) and Hassouna and Wareing (1964) reported total plant N gains in greenhouse experiments with *A. arenaria* grown in dune sand with an exogenous C source. They concluded that the N gains were due to N_2 fixation by *Azotobacter*. However, Ahmad and Neckelmann (1978) measured only low nitrogenase activity associated with roots of *A. arenaria* in unamended sand. Ralph (1978) measured higher nitrogenase activity in the rhizosphere than in the unexploited sand-dune soil associated with stands of *Ammophila breviligulata*. This higher level was thought to result from the activity of an unspecified *Azotobacter* isolated from the rhizosphere. Murphy (1975) measured low levels of acetylene reduction by anaerobic cultures of bacteria isolated from the rhizosphere of *Agropyron* spp. on Irish sand dunes. The results of experiments determining the effects of inoculation of other grasses with N_2-fixing

organisms have been mixed. Some researchers have concluded that plant growth increases were due to increases in plant-available N from asymbiotic N_2 fixation (Saric et al., 1987; Haahtela and Kari, 1986; Sarig et al., 1984; Lethbridge and Davidson, 1983; Abd-El-Malek, 1971). For the majority, however, the addition of an exogenous C source for the N_2-fixing microorganisms was necessary. Saric et al. (1987) report negative effects of inoculation with Azotobacter, Escherichia, Derxia, and Azospirillum spp. on N content of corn hybrids. Bacillus polymyxa, which fixes N_2 anaerobically (Grau and Wilson, 1962), has been isolated from the roots of field grown wheat (Lindberg and Granhall, 1984; Nelson et al., 1976) and a variety of native grasses in Oregon (Nelson et al., 1976). Wheat-root development in semi-solid agar was inhibited by inoculation by B. polymyxa although nitrogenase activity was recorded (Lindberg et al., 1985)

Many researchers have found significant enhancement of growth, but not plant-N content, due to root inoculation with asymbiotic N_2-fixing bacteria (Haahtela and Kari, 1986; Smith et al., 1976; Kapulnik et al., 1985; Lin et al., 1983; Brown, 1976). It has been suggested that growth increases are due to microbially-produced phytohormones which effected plant root morphology and nutrient uptake (Lin et al., 1983; Kapulnik et al., 1985; Brown, 1976).

The objective of this set of greenhouse experiments was to assess the effects of rhizosphere bacteria and N fertilization on growth and nutrition of sea oats in sand from the dunes of Anastasia State Recreation Area, FL.

Materials and Methods

Selection of Bacterial Strains for Inocula

For the first experiment, five bacterial isolates were chosen on the basis of relative N_2-fixing abilities (Sylvia and Will, 1988). Colonies selected from N-free, combined carbon (NFCC) media plates (Rennie, 1981) were tested for nitrogenase activity by the acetylene-reduction assay. Three milliliters of semi-solid NFCC media in a 10-mL tube were inoculated with 0.1 mL (10^7 CFU mL^{-1}) of a bacterial sample in log-phase growth and allowed to incubate at 35°C for 6 d. The tubes were then closed with serum stoppers and a 10% acetylene atmosphere was produced by removing 0.7 mL air from the tube using a gas-tight syringe and replacing it with 0.7 mL acetylene. The samples were incubated for an additional 2 h at 35°C before 0.5 mL of the tube atmosphere was removed and injected into a gas chromatograph (Varian Aerograph Model 940) fitted with a flame ionization detector and equipped with a 2.8 m column packed with Porapak R. Nitrogen was used as the carrier gas. The isolates selected for inoculation trials were (i) Bacillus polymyxa (identified by Microbial I.D., Inc., Newark, DE) isolated from the rhizosphere of sea oat plants from Atlantic Beach, FL, (ii) Alcaligenes denitrificans (identified by Microbial I.D., Inc.) isolated from the rhizosphere of sea oat plants from Miami Beach, FL, (iii) Azospirillum lipoferum strain sp USA5b (Pullman, WA USA/soil), (iv) Azospirillum brasiliense strain JM125A2 (Gainesville, FL USA/millet), (v) Klebsiella pneumoniae strain Beijing, and (vi) a combination of two non-N_2-fixing, Gram negative bacteria (T3E3 and T3E10) isolated from the

rhizosphere of sea oats in established dunes at Atlantic Beach, FL. The A. brasiliense ,A. lipoferum, and the K. pneumoniae isolates were identified by, and received from, J. Milam (Microbiology and Cell Science, U. of Fla., Gainesville) and were included to assess the effects of known N_2-fixing microbes on sea oat growth. For the second experiment the inoculants were K. pneumoniae and A. denitrificans, as described above.

For the third experiment, four bacterial isolates from the rhizosphere of sea oat plants in established dunes at Atlantic Beach, FL, were used as inoculants (#2, #16, #18, #19). These isolates tested negatively for N_2 fixation (see Results).

Greenhouse Culture Method

Sea oat seeds collected from plants on sand dunes at Anastasia State Recreation Area (ASRA), St. Augustine Beach, FL, were germinated and grown for 10 d in vermiculite, then transplanted to 620 cm^3 Deepot inserts (J.M. McConkey & Co, Inc., Sumner, WA) containing pasteurized (70°C for 4 h) ASRA sand (see Appendix A) at four seedlings per pot. Immediately prior to transplanting, 1 mL of a bacterial suspension (10^7 CFU mL^{-1}) or sterile water (control) was placed in the transplanting hole. The cultures had been grown in liquid nutrient broth to log phase, washed twice in NaCl (0.8%), and resuspended in sterile water. The plants were fertilized with 20 mL of a 0.1-strength modified Hoagland's solution (Appendix B) at the time of planting and every 14 d thereafter. One half of the plants in each inoculation treatment received N in the fertilizer solution at a rate of 2 mg L^{-1}, while the

other half received no fertilizer N. The pots were placed in a nonshaded greenhouse in a completely randomized block design. There were 10 replicates of each treatment in Experiments 1 and 2, and 8 replicates in Experiment 3. The duration and season of the experiments, and greenhouse light and temperature conditions, are given in Table 2-1.

Sampling and Analysis

The sea oats were harvested 63-70 d after transplanting. The number of plants which survived and the height of the tallest tiller in each pot were noted. Adhering sand was washed gently from the root mass in each pot before roots were weighed and subsampled (0.5 g) for total root length determination by the gridline-intersect method (Newman, 1966). Shoots were dried at 60°C for 48 h, weighed, and shredded by hand.

For P analysis, shoots were digested using the sealed-chamber method of Anderson and Henderson (1986), and P was determined on a Jarrel-Ash 9000 inductively-coupled argon plasma spectrometer (ICAP) at the University of Florida, Analytical Research Laboratory. For N analysis, shoots were digested by the Nelson and Sommers (1972) modification of the Kjeldahl method and analyzed for N on an Alpkem Rapid Flow Analyzer. The data were analyzed by ANOVA and differences in treatment means were evaluated by orthagonal contrasts ($P \leq 0.05$) (SAS Institute, Inc., 1985).

Table 2-1. Duration, season, and environmental conditions for greenhouse experiments.

Variables	EXPERIMENT 1	EXPERIMENT 2	EXPERIMENT 3
Duration (d)	63	70	63
Dates	3/86-5/86	10/86-1/87	5/86-7/86
Mean min.temp. (°C)	22	22	24
Mean max. temp. (°C)	33	29	36
Mean max. PPFD[a] (μmol m^{-2} s^{-1})	1232	1407	1183

[a] PPFD is photosynthetic photon flux density.

Results

Acetylene Reduction Assay

Measured ethylene production for K. pneumoniae, A. lipoferum, A. brasiliense, B. polymyxa, and A. denitrificans was 7.25, 14.18, 11.01, 0.11, and 0.07 nmol h^{-1}. There were no measurable quantities of ethylene produced by the samples designated #2, #16, #18, and #19.

Experiment 1

Fertilizer N resulted in significant increases ($P \leq 0.01$) in root dry mass (RDM), total root length (TRL), shoot dry mass (SDM), height of the tallest tiller (HTT), and percent and total shoot N and P (Tables 2-2 and 2-3). There was an interactive effect of N and microbial inoculation on the lower fertilizer-N level, plants inoculated with K. pneumoniae or T3E3+T3E10 had greater SDM compared to controls (40.6, 37.8 and 30.9 mg, At the higher fertilizer-N level, plants inoculated with K. pneumoniae had greater SDM compared to controls (152.9 and 85.8 mg, respectively). Control plants had higher percent shoot N than inoculated plants, regardless of N fertilization.

Nonsignificant trends in the data included increases in growth associated with bacterial inoculation. The ranges of values for growth and shoot nutrient content are given in Appendix C.

Experiment 2

Several of the trends observed in the initial experiment were also seen in this experiment. Nitrogen fertilization resulted in increases

Table 2-2. F values from ANOVA for Experiment 1.

Source	Root dry mass	Total root length	Height tallest tiller	Shoot dry mass	Shoot N %	Total shoot N	Shoot P %	Total shoot P
Microbial Inoculum (MI)	.0470	.1164	.3523	.0031	.0036	.0992	.0620	.8057
N fertilization (N)	.0001	.0001	.0001	.0001	.0144	.0001	.0001	.0001
MI*N	.1882	.7336	.3744	.0018	.3565	.7462	.1716	.0908
C.V. (%)	55	38	25	36	74	85	19	45
Error d.f.	85	88	90	89	69	69	42	42

Table 2-3. Main effects of N fertilization and root inoculation on growth of sea oat seedlings in ASRA sand for 63 d.

	Root dry mass (mg)	Total root length (cm)	Height tallest tiller (cm)	Shoot N (%)	Total shoot N (mg)	Shoot P (%)	Total shoot P (mg)
N rate (mg L^{-1})							
0	33.3 B[a]	299 B	16 B	0.87 B	0.28 B	0.22 A	0.08 B
2	128.4 A	602 A	29 A	1.29 A	1.31 A	0.11 B	0.13 A
Inoculation							
Control	69.1 A	-----	-----	1.96 A	-----	-----	-----
K. pneumoniae	102.8 B	-----	-----	0.62 B	-----	-----	-----
A. lipoferum	85.2 A	-----	-----	0.63 B	-----	-----	-----
A. brasilense	73.4 A	-----	-----	0.94 B	-----	-----	-----
B. polymyxa	75.6 A	-----	-----	1.21 B	-----	-----	-----
A. denitrificans	87.1 A	-----	-----	1.25 B	-----	-----	-----
T3E3+T3E10	75.7 A	-----	-----	0.97 B	-----	-----	-----

[a] Each value for shoot and root growth represents the mean of at least eight replicates, while values for nutrient contents represent the mean of four replicates. Means with the same letter within fertilizer treatments are not significantly different ($P \leq 0.05$). Microbial treatment means with the same letter as the Control mean are not significantly different from the Control mean ($P \leq 0.05$). Dashed lines indicate nonsignificant differences.

over controls in RDM, TRL, HTT, and SDM of 150, 73, 100, and 150%, respectively (Tables 2-4 and 2-5). Shoot-N concentrations and totals were increased by 90 and 500%, respectively. Percentage shoot P was reduced 28% by N fertilization; however, there was a 100% increase in total P. The nonsignificant increase in SDM associated with bacterial inoculation may have been responsible for the increased total shoot P.

Control plants had lower total shoot P than plants receiving bacterial inocula, regardless of N fertilization. Nonsignificant trends in the data included some increases in growth associated with bacterial inoculation. The mean values for growth and shoot-nutrient content are given in Appendix D.

Experiment 3

Nitrogen fertilization increased RDM, HTT, SDM, and total shoot N by 21, 22, 14, and 23%, respectively (Tables 2-6 and 2-7). Percent shoot N was decreased by inoculation with isolate #19, as compared to controls, regardless of N fertilization. There was an interaction between fertilizer-N level and bacterial inoculation with regard to shoot P (Tables 2-6 and 2-8). Percent and total shoot P were higher in plants receiving fertilizer N and inoculated with #16 than in the controls. Plants inoculated with #2 or #16 had increased total shoot P at the 0 mg L^{-1} fertilizer-N level, as compared to controls. As there were no trends among microbial treatments indicating beneficial or detrimental effects on growth, no further work was done with these non-N_2-fixing isolates.

Table 2-4. F values from ANOVA for Experiment 2.

Source	Root dry mass	Total root length	Height tallest tiller	Shoot dry mass	Shoot N %	Total shoot N	Shoot P %	Total shoot P
Microbial Inoculum (MI)	.1478	.1373	.2942	.3206	.1480	.8230	.2814	.1028
N fertilization (N)	.0001	.0001	.0001	.0001	.0001	.0001	.0045	.0001
MI*N	.2752	.2074	.2896	.2531	.1835	.6410	.1915	.1339
C.V. (%)	54	51	20	37	17	38	20	18
Error d.f.	46	43	46	46	12	12	12	12

Table 2-5. Main effects of N fertilization on growth of sea oat seedlings in ASRA sand for 70 d.

	Root dry mass (mg)	Total root length (cm)	Height tallest tiller (cm)	Shoot dry mass (mg)	Shoot N (%)	Total shoot N (mg)	Shoot P (%)	Total shoot P (mg)
N rate (mgL^{-1})								
0	18.3 B[a]	350 B	35 B	34.7 B	1.68 B	0.57 B	0.15 A	0.06 B
2	43.7 A	607 A	96 A	96.4 A	3.20 A	3.21 A	0.11 B	0.11 A

[a] Each value for shoot and root growth represents the mean of at least eight replicates, while values for nutrient contents represent the mean of three replicates. Means with the same letter treatments are not significantly different (P≤0.05).

Table 2-6. F values from ANOVA for Experiment 3.

Source	Root dry mass	Height tallest tiller	Shoot dry mass	Shoot N %	Total shoot N	Shoot P %	Total shoot P
Microbial Inoculum (MI)	.4109	.7634	.5738	.0258	.7185	.6001	.4862
N fertilization (N)	.0001	.0003	.0001	.1438	.0006	.0154	.0779
MI*N	.4498	.6351	.3505	.8653	.7530	.0292	.0041
C.V. (%)	24	14	23	13	23	13	21
Error d.f.	59	59	59	34	34	30	30

Table 2-7. Main effects of N fertilization and root inoculation with non-N_2-fixing bacteria on growth of sea oat seedlings in ASRA sand for 65 d.

	Root dry mass (mg)	Height tallest tiller (cm)	Shoot dry mass (mg)	Shoot N (%)	Total shoot N (mg)
N rate (mg L^{-1})					
0	30.2 B[a]	19 B	45.7 B	-----	0.48 B
2	38.4 A	22 A	58.4 A	-----	0.62 A
Inoculation					
Control	-----	-----	-----	1.05 A	-----
#2	-----	-----	-----	0.98 A	-----
#16	-----	-----	-----	1.07 A	-----
#18	-----	-----	-----	1.07 A	-----
#19	-----	-----	-----	0.89 B	-----

[a] Each value for shoot and root growth represents the mean of at least six replicates, while values for N content represent the mean of at least four replicates. Means with the same letter within fertilizer treatments are not significantly different ($P \leq 0.05$). Microbial treatment means with the same letter as the Control mean are not significantly different from the Control mean ($P \leq 0.05$). Dashed lines indicate nonsignificant differences.

Table 2-8. Effects of root inoculation with non-N_2-fixing bacteria on P content of sea oat seedlings in ASRA sand for 65 d, with and without N additions.

Inoculation treatment	Shoot P (%)	Total shoot P (mg)
	----------0 mg N L^{-1}----------	
Control	0.12 A[a]	0.03 A
#2	0.13 A	0.04 B
#16	0.14 A	0.04 B
#18	0.13 A	0.03 A
#19	0.13 A	0.03 A
	----------2 mg N L^{-1}----------	
Control	0.13 A	0.05 A
#2	0.12 A	0.04 A
#16	0.10 B	0.03 B
#18	0.11 A	0.05 A
#19	0.13 A	0.05 A

[a] Each value represents the mean of four replicates. Microbial treatment means with the same letter as the Control mean, within fertilizer treatment, are not significantly different from the Control mean ($P \leq 0.05$).

Discussion

Increases in growth due to N fertilization confirm that N is a limiting nutrient for growth of sea oats in the ASRA sand. That N is limiting for plant growth is consistent with the findings of Willis and Yemm (1961) and Hassouna and Wareing (1964) with native dune plants and A. arenaria, respectively. In the low N soil environment of the experiments reported herein, bacterial inoculation resulted in nonsignificant increases in RDM, TRL, and SDM. Variability in growth within varieties of wild grasses has been recognized as a problem in seed and forage production (Griffiths et al., 1980) and likely contributed to the variability seen in the growth of the sea oats in our experiments.

The fact that total and percent N in the inoculated plants tended to be lower than in control plants indicates that growth enhancement was not due to increased N availability by microbial N_2-fixation. The failure of K. pneumoniae, A. brasiliense, A. lipoferum, and B. polymyxa to increase plant-available N supports the idea of Barber and Lynch (1977) that root associated N_2-fixation in temperate climates is seriously limited by carbohydrate availability. This limitation is in agreement with the work by Lethbridge and Davidson (1983) in which the N content of wheat grown in sand and inoculated with several Azotobacter, Azospirillum, and Klebsiella spp. was not affected by the inoculation unless a carbohydrate (glucose or malate) was added. In a greenhouse study in which Hassouna and Wareing (1964) inoculated A. arenaria roots growing in sand with a mixture of A. arenaria rhizosphere microorganisms

(including Azotobacter), the authors found no effect of bacterial inoculation on growth when no nutrients were added. They suggest that carbohydrates and possibly P sources were limiting N fixation.

Decreased N content may have resulted from competition for the limited N available in the soil. Work with excised roots of Zea mays L. (Pioneer Hybrid 3320) (Lin et al., 1983) inoculated with A. brasiliense, indicates the increased SDM was probably due to enhanced mineral uptake caused by the rhizosphere bacterial population. Although the mechanism by which nutrient uptake is increased is not known, the authors suggest that hormones produced by the rhizosphere bacteria may be affecting root morphology and function in nutrient uptake. The bacterial inoculum associated with the greatest increases in shoot and root growth in the present study (i.e. K. pneumoniae and A. denitrificans) also resulted in the greatest increases (nonsignificant) in total shoot P. This association between P uptake and growth suggests that P is a limiting nutrient and its uptake or availability is enhanced by these bacteria. Although of a genus common in soils, A. denitrificans has not been reported to affect plant growth.

CHAPTER 3
INCREASED AVAILABILITY OF PHOSPHORUS TO *UNIOLA PANICULATA* DUE TO BACTERIAL ROOT INOCULATION

Introduction

There has long been an interest in the ability of rhizosphere bacteria to solubilize sparingly-soluble (or "insoluble") P sources. Work with plants grown in sterile media with and without rhizosphere bacteria indicated that bacteria caused an increase in dry matter production and uptake of sparingly-soluble P. Oats inoculated with rhizosphere bacteria and grown in media to which $CaHPO_4$ was added as the only P source had increased P content of 16 to 54% compared to control plants without bacteria (Gerretsen, 1948). Over 100 bacterial isolates from the rhizosphere of oats were found by Louw and Webley (1959) to solubilize several forms of insoluble Ca-phosphate minerals *in vitro*. The majority of the isolates produced Ca-chelating lactic and 2-ketogluconic acids from glucose. The ability to dissolve phosphate minerals was also found for rhizosphere bacteria from wheat (Katznelson and Bose, 1959) and barley (Katznelson et al., 1962). These authors suggested that solubilized P would be available to the plant.

Barea et al. (1976) found that phosphate-solubilizing rhizosphere bacteria from several genera had the ability to produce plant growth regulators. They concluded that the effects on plant growth reported

due to inoculation with such organisms were primarily the result of the growth regulators; increased plant growth would result in increased P uptake, possibly from a pool provided by the phosphate-dissolving bacteria.

The ability of rhizosphere organisms to provide plant-available P from an insoluble source is relevant to the majority of east coast Florida dune sands where plant-available P is low. These sands are not uniform; as differences in chemical properties exist. For example, the ASRA sand used in the previous experiments has higher NO_3-N, water-extractable P, and double-acid (Mehlich-I) extractable P than the Miami Beach sand (Appendix A). The mineralogy of the fine silt and clay fraction of the ASRA sand was found to be dominated by a brushite-like ($CaHPO_4 \cdot 2H_2O$) compound (W.G. Harris, personal communication). Although the X-ray spacing of the hydrated mineral was similar to brushite, the infrared spectrophotometry patterns were not. It is possible that some other compound(s) was masking the presence of brushite. Brushite forms stable crystals which are relatively insoluble in water, but soluble in weak acids (Lehr et al., 1967), such as the organic acids exuded by bacteria and plant roots. This brushite-like mineral, therefore, was probably the source of the majority of the acid-soluble P in the ASRA sand and may be, at least partially, available to plants and bacteria. Further work is needed to characterize the P-containing mineral present in ASRA sand and identify the source from which it is formed.

Three greenhouse experiments were established to assess the effects of root inoculation with several phosphate-solubilizing bacterial

isolates on availability of readily- and sparingly-soluble P to, and growth characteristics of, sea oats grown in ASRA or Miami Beach sand.

Materials and Methods

The first greenhouse experiment was established to evaluate uptake of readily-soluble P. Sea oat seeds were germinated and grown for 10 d in vermiculite, then transplanted (three seedlings per pot) to 620 cm^3 Deepots inserts containing pasteurized ASRA sand (70°C for 4 h). Immediately prior to transplanting, 1 mL (10^7 CFU mL^{-1}) of a bacterial suspension or sterile water (controls) was placed in the transplanting hole. The strains used were K. pneumoniae strain Beijing, A. lipoferum strain sp USA5b, and A. denitrificans as described in Chapter 1. The cultures were prepared for use as inoculum as described in Chapter 1. Plants were fertilized with 20 mL of a 0.1-strength modified Hoagland's solution (Appendix B) containing either 0 or 0.2 mg P L^{-1} at the time of planting and every 14 d thereafter. The nitrogen content of the fertilizer was 2 mg L^{-1} for the first 60 d, and at 20 mg L^{-1} for the remaining 30 d. The pots were placed in a nonshaded greenhouse and during the final 30 d of growth they received 900 μmol m^{-2} s^{-1} of supplemental light for 14 h per d (7 AM-9 PM) from metal halide lamps. Temperature at plant height under the lamps was 31°C. The experiment was arranged in a completely randomized block design with seven replicates of each treatment. The sea oats were harvested after 90 d and analyzed as described in Chapter 1.

The second greenhouse experiment was established to evaluate the uptake of relatively insoluble P ($CaHPO_4$) in ASRA sand. This experiment was set up as described above, under natural light conditions in the greenhouse, using the K. pneumoniae, A. lipoferum, and A. denitrificans strains. Pots contained pasteurized ASRA sand with 50 kg P ha^{-1} as $CaHPO_4$ mixed in by hand prior to planting. Plants were fertilized with 20 mL of a 0.1-strength modified Hoagland's solution containing 0 mg P L^{-1} at the time of planting and every 14 d thereafter. The sea oats were harvested after 90 d and analyzed as described in Chapter 1.

The third greenhouse experiment was established to evaluate the uptake of P from a relatively insoluble source ($CaHPO_4$) in Miami Beach sand. This experiment was set up and fertilized as described above for Experiment 2. Pots contained pasteurized Miami Beach sand (see Appendix A) with 50 kg P ha^{-1} as $CaHPO_4$ mixed in by hand.

The duration and season of the experiments, and greenhouse light and temperature conditions are given in Table 3-1. The sea oats were harvested and analyzed as described in Chapter 1.

A laboratory study was conducted to determine the ability of the K. pneumoniae, A. lipoferum, and A. denitrificans strains to solubilize $CaHPO_4$ in vitro. Fifteen milliliters of sterile $CaHPO_4$-containing medium was inoculated with 1 mL of a bacterial suspension (10^7 CFU). The cultures were prepared for use as inoculum as described above. The medium contained in mg L^{-1}: 2000, sucrose; 10, $CaCl_2$; 200, $MgSO_4 \cdot 7H_2O$; 20, $NaMoO_4$; 250, KNO_3; 160, NH_4NO_3; 5.6, Fe(EDTA); 1000, $CaHPO_4$, and 10 g Difco agar. Plates (8 per bacterial strain) were observed at 24-h for

Table 3-1. Duration, season, and environmental conditions for greenhouse experiments.

Variables	EXPERIMENT 1	EXPERIMENT 2	EXPERIMENT 3
Duration (d)	90	90	100
Dates	9/86-12/86	6/87-9/87	8/87-12/87
Mean min. temp. (°C)	22	23	19
Mean max. temp. (°C)	31	31	27
Mean max. PPFD[a] (μmol m^{-2} s^{-1})	1475	1766	1285

[a] PPFD is photosynthetic photon flux density

10 d intervals for zones of clearing around the bacterial colonies indicating $CaHPO_4$ solubilization (Katznelson et al., 1962).

Results

Effect of Soluble P Source

The level of P fertilization had no effect on RDM, TRL, HTT, SDM, or shoot N (Table 3-2). Phosphorus fertilization did increase percent and total shoot P by 27 and 58%, respectively (Table 3-3). Bacterial inoculation had no significant effect on the measured growth parameters. It is interesting to note, however, that under the 2 mg P L^{-1} fertilizer regime, bacterial inoculation was associated with nonsignificant decreases in shoot N, and nonsignificant increases in RDM, TRL, HTT, SDM, and shoot P. There data are presented in Appendix E.

Effect of Insoluble P Source

Inoculation with K. pneumoniae resulted in increases in RDM, TRL, HTT, and SDM of 6, 33, 43, 6, and 23%, respectively, over controls (Tables 3-4 and 3-5) in ASRA sand. Shoot P and N were not affected by inoculation (see Appendix F).

Microbial root inoculation had no significant effect on RDM, TRL, HTT, SDM, total shoot N, percent shoot P, and total shoot P of plants grown in Miami Beach sand (Table 3-6). All microbial treatments increased RDM, TRL, HTT, and SDM to some degree, although not significantly (see Appendix G). Inoculation with K. pneumoniae or A. denitrificans decreased percent shoot N by 32 and 29%, respectively, from the control value of 1.01%.

Table 3-2. F values from ANOVA for Experiment 1.

Source	Root dry mass	Total root length	Height tallest tiller	Shoot dry mass	Shoot N %	Total shoot N	Shoot P %	Total shoot P
Microbial Inoculum (MI)	.2622	.1260	.4313	.3199	.6296	.6936	.1149	.1671
P fertilization (P)	.6398	.3335	.1084	.1586	.3981	.5467	.0001	.0188
MI*P	.7924	.2766	.1423	.1366	.0788	.3563	.5834	.2921
C.V. (%)	91	68	38	54	23	41	18	63
Error d.f.	42	42	42	42	16	16	40	40

Table 3-3. Main effect of P fertilization on shoot-P content of sea oat seedlings in ASRA sand for 90 d.

	Shoot P (%)	Total shoot P (mg)
P rate (mg L^{-1})		
0	0.05 B	0.04 B
2	0.07 A	0.07 A

[a] Each value represents the mean of at least eight replicates. Means with the same letter are not significantly different ($P \leq 0.05$).

Table 3-4. F values from ANOVA for Experiment 2.

Source	Root dry mass	Total root length	Height tallest tiller	Shoot dry mass	Shoot N %	Total shoot N	Shoot P %	Total shoot P
Microbial Inoculum (MI)	.0004	.0001	.0528	.0001	.9160	.2862	.6096	.1274
C.V. (%)	22	32	9	15	13	10	20	24
Error d.f.	74	74	74	74	8	8	20	20

Table 3-5. Effect of bacterial root inoculation on growth of sea oat seedlings in ASRA sand, amended with $CaHPO_4$, for 90 d.

Inoculation Treatment	Root dry mass (mg)	Total root length (cm)	Height tallest tiller (cm)	Shoot dry mass (mg)
Control	70.0 A[a]	386 A	29 A	134.8 A
K. pneumoniae	93.1 B	552 B	30 B	165.8 B
A. lipoferum	76.1 A	372 A	29 A	132.4 A
A. denitrificans	76.5 A	378 A	28 A	143.2 A

[a] Each value represents the mean of at least 19 replicates. Microbial treatment means with the same letter as the Control mean are not significantly different from the Control mean ($P \leq 0.05$).

Table 3-6. F values from ANOVA for Experiment 3.

Source	Root dry mass	Total root length	Height tallest tiller	Shoot dry mass	Shoot N %	Total shoot N	Shoot P %	Total shoot P
Microbial Inoculum (MI)	.0736	.1931	.1266	.3242	.0451	.9291	.4230	.7349
C.V. (%)	44	32	14	23	19	48	38	32
Error d.f.	28	28	28	28	11	11	10	10

$CaHPO_4$ Solubilization in vitro

Zones of $CaHPO_4$ solubilization were visible around the colonies of all three of the bacterial strains within 96 h of plate inoculation. Klebsiella pneumoniae grew rapidly and had clear zones about 4-mm wide around the colonies. Azospirillum lipoferum and A. denitrificans colonies were surrounded by 1-2 mm, and less than 1-mm wide clear zones, respectively. Growth and $CaHPO_4$ solubilization continued throughout the 8-d observation period.

Discussion

In this set of experiments, the amount of soluble P added to the ASRA sand did not have an effect on any growth parameters measured except shoot P. The nonsignificant increase in P uptake and growth due to inoculation with A. lipoferum under conditions where no soluble P was added may have been the result of one or more processes. Bacterially-induced root hair production in wheat and millet following inoculation with A. brasiliense has been noted (Kapulnik et al., 1985; Tien et al., 1979, respectively). This species has been shown to produce IAA, cytokinins, and gibberillins in vitro which may induce root-hair formation (Jain and Patriquin, 1984; Tien et al., 1979). It was hypothesized that additional root hairs would allow exploitation of larger volumes of soil for soluble P. In addition, organic acids excreted by a large root mass may increase the amount of relatively insoluble P, such as the brushite-like mineral in the ASRA sand, made available to the plants. The increased volume of rhizosphere also

provides a larger habitat for A. lipoferum and other bacteria which have been shown to have the capacity to solubilize relatively-insoluble P.

When a relatively insoluble P source was added to the ASRA sand, K. pneumoniae increased plant growth, but not P uptake. Phosphorus uptake was greater with the addition of $CaHPO_4$, as compared to when no soluble-P was added in the first experiment, indicating that the P was becoming available to the plant. Root-induced lowering of the rhizosphere pH or root-produced P-chelating organic acids may have been responsible for making P in the $CaHPO_4$ available to the plants (Marschner et al., 1986).

When insoluble P was added to the Miami Beach sand, with its lower water-soluble and acid-extractable P contents, all bacterial inoculation treatments were associated with nonsignificant increases in plant growth. The more extensive bacterially-induced growth enhancement, as compared to in the ASRA sand, likely was due to the lower nutrient content of the Miami Beach sand and resulted from one or more of the mechanisms mentioned previously. The fact that P uptake in the shoots was not affected by inoculation, however, indicates that a mechanism other than a bacterially-induced, increased P availability to plants was responsible. It is possible that P was being sequestered in the increased root mass of plants inoculated with bacteria. Root sample sizes were too small to perform nutrient analyses.

The results of these experiments indicate that, under the experimental conditions, bacterial root inoculation resulted in enhancement, nonsignificant for the most part, growth but not P uptake to the shoots in dune sands where P was supplied in either soluble or insoluble form. The reasons for this increased growth require further study.

CHAPTER 4
GROWTH ENHANCEMENT OF UNIOLA PANICULATA RESULTING
FROM DUAL INOCULATION WITH BACTERIA AND MYCORRHIZAL FUNGI

Introduction

Specific strains of bacteria and fungi have been shown to increase growth of dune grasses (Sylvia and Burks, 1988; Abdel Wahab and Wareing, 1980; Nicolson and Johnston, 1979) and other plants (Smith et al., 1986; Kapulnik et al., 1985). Studies on the effects of dual inoculation with a VAM fungus and a bacterial strain have focused mainly on such functional groups as N_2-fixing, or phosphate-solubilizing bacteria.

Barea et al. (1983) inoculated the roots of greenhouse-grown maize and ryegrass with strains of A. brasiliense and Glomus mosseae Gerdemann and Trappe. Although A. brasiliense increased the level of VAM infection, the effects of inoculation on plant growth were variable and generally nonsignificant. Pacovsky et al. (1985) found the growth and N content of sorghum to be increased by dual inoculation with A. brasiliense and Glomus fasciculatum (Thaxter sensu Gerd.) Gerdemann and Trappe; total plant response could be accounted for by adding the VAM and bacteria effects. They also found fungal colonization and biomass were increased by A. brasiliense. Rao et al. (1985) found no significant increase in VAM colonization or plant dry matter and grain yield in barley inoculated with G. mosseae in combination with A.

brasiliense. Bagyaraj and Menge (1978) found tomato-root infection by G. fasciculatum was increased by Azotobacter chroococcum Beijerinck. Plant dry weight was increased by dual inoculation as compared to noninoculated plants. Tomato-seedling roots inoculated with unidentified phosphate-rock-dissolving bacteria (PRDB), with or without the VAM fungus Gigaspora margarita Becker and Hall, had no effect on dry mass yield or P uptake (Lee and Bagyaraj, 1986). When rock phosphate was added to this low-P soil. inoculation with G. margarita, with or without the PRDB, resulted in increased dry matter yield and P uptake.

Azcon et al. (1976) studied the effects of dual inoculation of lavender (Lavandula officinalis) with a VAM fungus (G. fasciculatum or G. mosseae) and a PRDB that produced plant-growth regulators (two Pseudomonas spp. and one Agrobacterium sp.) in a low P, rock-phosphate-amended soil. Plants inoculated with any combination of one fungus and one bacterium had higher total P than uninoculated plants or those inoculated with a fungus or bacteria alone. In another study with these organisms in rock-phosphate-amended soil, Barea et al. (1975) found that inoculation with the VAM fungus enhanced population maintenance of the bacteria in the rhizospheres of lavender and maize. The bacteria did not influence the level of VAM fungal root infection in either plant. Inoculation with the fungus or bacteria alone or in combination generally had no effect on growth or P uptake. Raj et al. (1981) inoculated finger millet with G. fasciculatum and phosphate-dissolving Bacillus circulans in a tricalcium phosphate-amended soil. Total shoot P was higher in plants inoculated with both organisms than in any other treatment.

Meyer and Linderman (1986) found dual inoculation of subterranean clover with an unidentified VAM fungus and a plant-growth-promoting bacterium, Pseudomonas putida, to increase root and shoot dry mass. Although initially increased by P. putida, VAM fungal colonization was subsequently not affected by the bacteria. Mosse (1962) found that infection of several plant types grown in a N-deficient inorganic salts medium did not occur unless a Pseudomonas sp. was also added. She suggested that bacterial metabolite(s) acted on the root-cell wall to modify or dissolve its pectic components and increase its susceptibility to fungal infection.

Greenhouse experiments were established to assess the effects of root inoculation with several bacterial isolates alone or in combination with a VAM fungal inoculum that consisted of a mixture of Glomus deserticola Trappe, Bloss and Menge (isolate 305) and Glomus macrocarpum Tul. and Tul. (isolate S328), on growth and nutrient status of sea oats in two Florida east coast beach dune sands.

Materials and Methods

Selection of Bacterial and Fungal Inocula

The K. pneumoniae, A. lipoferum, and A. denitrificans strains are the same as described in Chapter 1. The VAM fungal isolates were isolated from the rhizosphere of sea oats on established primary dunes at Anastasia State Recreation Area at St. Augustine Beach, FL, by D. M. Sylvia. G. deserticola was found to be, overall, the most abundant species at ASRA and in established dunes at Atlantic Beach, FL (Sylvia,

1986; Sylvia and Will, 1988). Preliminary work indicated that G. deserticola is a successful colonizer of sea oats and serves to enhance the plant's growth in the greenhouse and field (Sylvia and Burks, 1988; Sylvia, in review). The VAM fungi were maintained in pot cultures of sea oats in pasteurized beach sand.

Greenhouse Culture Method

Experiments 1, 2, and 3. Glomus deserticola spores were collected from pot cultures by wet sieving and sucrose centrifugation (Gerdemann and Nicolson, 1963). Ten days prior to planting, approximately 50 spores in 2-mL of sterile water were placed at a depth of 5 cm from the top of Deepots containing 600 cm^3 of pasteurized ASRA sand (70°C for 4 h). Control pots received 2 mL of water used to wash the spores on the 45-μm screen from which the spores were collected. Sea oat seeds were germinated and grown for 10 d in vermiculite, then transplanted to the Deepots containing sand and spores (three seedlings per pot). Immediately prior to transplanting, 1 mL (10^7 CFU mL^{-1}) of a bacterial suspension or sterile water (controls) was placed in the transplanting hole as follows: Experiment (1) K. pneumoniae; Experiment (2) A. denitrificans; Experiment (3) K. pneumoniae or A. denitrificans. The cultures were prepared for use as inocula as described in Chapter 1. Plants were fertilized with 20 mL of a 0.1-strength modified Hoagland's solution (Appendix B) at the time of planting and every 14 d thereafter. The plants in Experiments 1 and 2 received N at a rate of 20 mg L^{-1} and P at 0.05 mg L^{-1}. Plants in Experiment 3 received N at a rate of 10 mg L^{-1} and P at either 0.03 or 0.3 mg L^{-1}. The pots were placed in a

nonshaded greenhouse in a completely randomized block design. There were ten replicates of each treatment in Experiments 1, and 2, and 15 replicates in Experiment 3.

Experiment 4. Pots contained either ASRA or Miami Beach pasteurized sand (see Appendix A for chemical properties) and received 20 cm of washed, chopped, sea-oat-root segments. The roots were obtained from greenhouse pot cultures of D. M. Sylvia and had 10% of their root length colonized with G. deserticola and G. macrocarpum. The seedlings were grown for 21 d in a potting mix of vermiculite and peat by a commercial grower (Otto Bundy, Horticultural Systems, Inc., Parrish, FL), and then transplanted, two per pot, to the experimental containers. A culture, containing approximately equal proportions of K. pneumoniae and A. lipoferum, was grown and used for inoculum as described above. Plants received N at a rate of 10 mg L^{-1} and P at either 0 or 0.05 mg L^{-1}. The pots were placed in a nonshaded greenhouse in a completely randomized block design with ten replicates of each treatment. The greenhouse light and temperature conditions, and dates of the experiments are given in Table 4-1.

Sampling and Analysis

The sea oats were harvested after 100-d growth in Deepots and analyzed as described in Chapter 1. The portion of the root mass subsampled for VAM fungal colonization was cleared in 10% KOH overnight and stained with 0.05% trypan blue. Root length colonized by VAM fungi was estimated by the gridline-intersect method (Newman, 1966). Percentage data were subjected to an arcsine transformation for analysis.

Table 4-1. Duration, season, and environmental conditions for greenhouse experiments.

Variables	EXPERIMENT 1	2	3	4
Duration (d)	100	100	100	100
Dates	10/86-1/87	11/86-2/87	4/87-7/87	8/87-12/87
Mean min. temp. (°C)	22	22	23	19
Mean max. temp. (°C)	30	28	30	27
Mean max. PPFD[a] (μmol m^{-2} s^{-1})	1431	1470	1792	1285

[a] PPFD is photosynthetic photon flux density.

Results

Spores as Inoculum

There were no differences among treatments in RDM, TRL, HTT, SDM, and shoot P in Experiment 1 (Table 4-2). Plant-N data were insufficient for statistical analysis. All data means are given in Appendix H. A nonsignificant increase in shoot P was associated with inoculation with the VAM fungi spores alone. Inoculation with K. pneumoniae was associated with nonsignificant increases in RDM and TRL.

There were no differences among treatments with regard to RDM, TRL, HTT, SDM, and total shoot P in Experiment 2 (Table 4-3). Plant-N data were insufficient for statistical analysis. All data means are given in Appendix I. Percent P in shoots of plants receiving VAM fungal spores alone or in combination with A. denitrificans was increased over that of control plants. Percent colonization remained fairly low in those treatment receiving the VAM fungi and was not affected by the presence of the bacteria.

In Experiment 3, no colonization was evident in the roots of plants inoculated with VAM fungal spores. The higher P-fertilization level resulted in increases in HTT and SDM of 11 and 14%, respectively (Tables 4-4 and 4-5). Inoculation with K. pneumoniae resulted in increases of 23, 34, and 20% in RDM, TRL, and SDM, respectively, regardless of fertilizer-P level, over controls (113.2 mg, 349 cm, and 172.4 mg, respectively). There was an interactive effect of microbial inoculation and P-fertilizer level on percent shoot N. At the lower P-fertilization level, inoculation with K. pneumoniae, A. denitrificans/VAM fungi, and

Table 4-2. F values from ANOVA for Experiment 1.

Source	Root dry mass	Root colonized (%)	Total root length	Height tallest tiller	Shoot dry mass	Shoot P %	Total shoot P
Microbial Inoculum (MI)	.9951	.0652	.9010	.3852	.8901	.1138	.2809
C.V. (%)	47	92	20	40	63	28	60
Error d.f.	34	18	33	34	34	31	31

Table 4-3. F values from ANOVA for Experiment 2.

Source	Root dry mass	Root colonized (%)	Total root length	Height tallest tiller	Shoot dry mass	Shoot P %	Total shoot P
Microbial Inoculum (MI)	.7435	.4430	.6143	.7533	.6077	.0067	.4951
C.V. (%)	46	47	28	36	14	30	45
Error d.f.	32	13	32	32	32	26	26

Table 4-4. F values from ANOVA for Experiment 3.

Source	Root dry mass	Total root length	Height tallest tiller	Shoot dry mass	Shoot N %	Total shoot N	Shoot P %	Total shoot P
Microbial Inoculum (MI)	.0491	.0005	.1203	.0003	.2606	.7787	.4545	.1890
P fertilization (P)	.1289	.5736	.0066	.0173	.1784	.3229	.2409	.9691
MI*P	.1249	.3349	.0898	.9230	.0389	.4776	.9717	.8343
C.V. (%)	25	31	11	18	12	18	20	27
Error d.f.	162	162	162	162	24	24	60	60

Table 4-5. Main effects of P fertilization and root inoculation with bacteria and a mixture of G. deserticola and G. macrocarpum spores (VAM) on growth of sea oat seedlings in ASRA sand for 100 d.

	Root dry mass (mg)	Total root length (cm)	Height tallest tiller (cm)	Shoot dry mass (%)
P rate (mg L^{-1})				
0.03	-----	-----	32 B[a]	188.3 B
0.30	-----	-----	34 A	201.7 A
Inoculation				
Control	113.2 A	349 A	-----	172.4 A
VAM	126.8 A	340 A	-----	186.3 A
K. pneumoniae	139.4 B	469 B	-----	214.2 B
A. denitrificans	120.7 A	376 A	-----	192.1 B
A. denitrificans-VAM	127.3 A	353 A	-----	195.0 B
K. pneumoniae-VAM	130.1 B	400 A	-----	208.7 A

[a] Each value represents the mean of at least eight replicates. Means with the same letter within fertilizer treatments are not significantly different ($P \leq 0.05$). Microbial treatment means with the same letter as the Control mean are not significantly different from the Control mean ($P \leq 0.05$). Dashed lines indicate nonsignificant differences.

K. pneumoniae/VAM fungi decreased percent shoot N, as compared to controls (1.29, 1.30, 1.36, and 1.67%, respectively). At the higher P-fertilization level, inoculation with *A. denitrificans* increased percent shoot N over controls (1.59 and 1.25%, respectively). Inoculation with *K. pneumoniae* was associated with nonsignificant increases in RDM and TRL at the lower fertilizer-P level, and nonsignificant increases in TRL, SDM, and percent shoot P at the higher fertilizer-P level.

Root-Hyphae-Spores as Inoculum

A portion of the plants in Experiment 4 were found to be contaminated with *Glomus occultum* Walker sp. nov. This problem was most pronounced in plants grown in the Miami Beach sand where eight control pots and 11 pots of plants inoculated with the bacteria alone were found to contain *G. occultum*. Only two of the control pots of ASRA sand were found to be contaminated. All contaminated pots were excluded from analysis. *Glomus occultum* produced hyaline spores outside of the root which were very distinct from the reddish brown to brown, mainly internal spores of *G. deserticola* and *G. macrocarpum*. Close inspection of the roots of plants inoculated with the VAM fungi produced no evidence of *G. occultum* colonization.

Miami Beach sand. Phosphorus fertilization increased RDM by 88% (Tables 4-6 and 4-7). The VAM fungi colonization in plants receiving P was increased by the combined bacterial and fungal inoculation as compared to fungal inoculation alone (56 and 41%, respectively). A nonsignificant increase over controls in colonization of plants not

Table 4-6. F values from ANOVA for Experiment 4. Miami Beach sand.

Source	Root dry mass	Total root length	Root colonized (%)	Height tallest tiller	Shoot dry mass	Shoot N %	Total shoot N	Shoot P %	Total shoot P
Microbial Inoculum (MI)	.1665	.2363	.8177	.0961	.1565	-----	-----	.0139	.0011
P fertilization (P)	.0489	.1348	.4060	.1764	.0940	.0343	.8871	.8434	.9492
MI*P	.2481	.5025	.0271	.3357	.2567	-----	-----	.5718	.1577
C.V. (%)	89	78	44	48	61	13	35	33	55
Error d.f.	34	34	23	34	34	16	16	24	24

Table 4-7. Main effects of P fertilization and root inoculation with bacteria and a mixture of G. deserticola and G. macrocarpum root-hyphae-spore inoculum (VAM) on growth of sea oat seedlings grown in Miami Beach sand for 100 d.

	Root dry mass (mg)	Shoot N (%)	Total shoot N (mg)	Shoot P (%)	Total shoot P (mg)
P rate (mg L^{-1})					
0	18.2 B[a]	1.43 B	-----	-----	-----
0.05	34.6 A	1.58 A	-----	-----	-----
Inoculation					
Control	-----	-----	-----	0.08 A	0.09 A
VAM	-----	-----	-----	0.11 A	0.13 A
K. pneumoniae/ A. lipoferum	-----	-----	-----	0.16 B	0.32 B
VAM/K. pneumoniae/ A. lipoferum	-----	-----	-----	0.12 B	0.15 A

[a] Each value for growth and P represents the mean of at least four replicates, while values for N represent three replicates. Means with the same letter within fertilizer treatments are not significantly different ($P \leq 0.05$). Microbial treatment means with the same letter as the Control mean are not significantly different from the Control mean ($P \leq 0.05$). Dashed lines indicate nonsignificant differences.

receiving P was associated with bacterial inoculation (32 and 53%, respectively). The G. occultum contamination of control plants grown in Miami Beach sand made comparisons of percent and total shoot N among microbial inoculum treatments and controls impossible. Among inoculated plants, P fertilization resulted in an 10% increase in percent shoot N over controls (Table 4-7). Regardless of fertilizer P level, percent and total shoot P were increased by inoculation with the bacterial combination as compared to controls. Root inoculation was associated with several nonsignificant increases in growth, therefore, the means of the data are given in Appendix J.

ASRA sand. Fertilization with P resulted in increases of 28, 37, 29 19, and 30% in percent root colonized, RDM, TRL, HTT, and SDM, respectively (Tables 4-8 and 4-9). Inoculation with VAM fungi resulted in increased RDM over controls, regardless of P-fertilization level.

Control plants receiving no fertilizer P had higher percent shoot N (1.42%) than plants inoculated with the bacteria alone (1.03%), the fungi alone (0.99%), or the combination (1.07%). Plants inoculated with the bacteria and fungi in combination had increased % shoot N, as compared to controls (1.23 and 0.83%, respectively). Inoculation with either the bacteria alone or in combination with the VAM fungi resulted in higher total shoot N compared to control plants, regardless of fertilizer-P level (Table 4-9). It is interesting to note that under the 0.05 mg P L^{-1} fertilizer regime especially, bacterial inoculation and VAM fungi inoculation were associated with nonsignificant increases in RDM, TRL, HTT, SDM, and shoot P. These data are presented in Appendix K.

Table 4-8. F values from ANOVA for Experiment 4. ASRA sand.

Source	Root dry mass	Total root length	Root colonized (%)	Height tallest tiller	Shoot dry mass	Shoot N %	Total shoot N	Shoot P %	Total shoot P
Microbial Inoculum (MI)	.0071	.2510	.6200	.0962	.8361	.4545	.0162	.1184	.4304
P fertilization (P)	.0229	.0348	.0386	.0004	.0057	.1924	.2319	.9953	.3318
MI*P	.5708	.5562	.3327	.9517	.4558	.0187	.2534	.3565	.4351
C.V. (%)	58	50	35	20	39	18	26	41	42
Error d.f.	64	64	33	64	64	16	16	24	24

Table 4-9. Main effects of P fertilization and root inoculation with bacteria and a mixture of G. deserticola and G. macrocarpum root-hyphae-spore inoculum (VAM) on growth of sea oat seedlings grown in ASRA sand for 100 d.

	Root dry mass (mg)	Total root length (cm)	Root colonized (%)	Height tallest tiller (cm)	Total shoot N (mg)
P rate (mg L^{-1})					
0	57.2 B[a]	311 B	35 B	27 B	0.18 B
0.05	42.4 A	402 A	42 A	33 A	0.20 A
Inoculation					
Control	45.8 A	-----	-----	-----	1.19 A
VAM	64.2 B	-----	-----	-----	1.45 A
K. pneumoniae/ A. lipoferum	58.2 A	-----	-----	-----	1.71 B
VAM/K. pneumoniae/ A. lipoferum	37.5 A	-----	-----	-----	2.17 B

[a] Each value for growth represents the mean of at least seven replicates, while values for root colonization represent at least four replicates and values for N represent three replicates. Means with the same letter within fertilizer treatments are not significantly different ($P \leq 0.05$). Microbial treatment means with the same letter as the Control mean are not significantly different from the Control mean ($P \leq 0.05$). Dashed lines indicate nonsignificant differences.

Discussion

In this set of experiments, there was no consistent evidence of a synergistic effect of dual inoculation of K. pneumoniae or A. denitrificans with the VAM fungi on sea oat growth. The positive effect of colonization of roots by VAM fungi on plant-P uptake was seen in treatments receiving fertilizer P. The increase in shoot P is consistent with the results of Sylvia and Burks (1988) with this G. deserticola isolate. As found in the experiments described in Chapter 1, there was no evidence of significant plant-N increases due to inoculation with the two known N_2-fixing bacteria.

Root colonization by VAM fungi was lower in experiments using spores alone than when a root-hyphae-spore inoculum was used. This is because the latter contains fungal propagules other than spores which allow a more rapid formation of mycorrhizae (Hepper and Smith, 1976). It is also possible that one or more germination factors required for breaking dormancy were associated with the hyphae and roots. Other factors affecting spore germination are noted in Chapter 5. The presence of the bacterial inoculum had no effect on colonization of roots by VAM fungi when spore inoculum was used. These bacteria did not appear to be enhancing spore germination as suggested by Mugnier and Mosse (1987) for the effect of Streptomyces orientalis on Glomus mosseae Rothamsted isolate. On the other hand, colonization was enhanced when a root-hyphae-spore inoculum was used in the Miami Beach sand. These findings are in agreement with the results of Rao et al. (1985) with G. mosseae, A. brasiliense, and barley, Pacovsky et al. (1985) with G.

fasciculatum, A. brasiliense, and sorghum, and others. The bacteria may have affected the plant-root-cell wall thereby affecting the susceptibility of the plant tissue to fungal attack. Azospirillum brasiliense has been shown to produce pectolytic enzymes in vitro which may act to soften plant-root-cell walls in the soil (Umali-Garcia et al., 1980). This mechanism was suggested by Mosse (1962) as the explanation for increased colonization of roots of several plants by an Endogone sp. in the presence of a Pseudomonas sp.. It was also suggested by Meyer and Linderman (1986) to explain enhanced colonization of clover roots by an unidentified VAM fungus in the presence of Pseudomonas putida. As the fungal inoculum consisted of only spores in our experiment, an effect of the bacteria on spore germination can not be ruled out entirely. Further study is required to understand the role of bacteria in co-inoculation experiments with VAM fungi.

CHAPTER 5
ENHANCEMENT OF VAM FUNGI SPORE
GERMINATION BY KLEBSIELLA PNEUMONIAE

Introduction

Germination of spores and establishment of root infection using spores alone under aseptic or greenhouse conditions are difficult with some strains of VAM fungi (Mugnier and Mosse, 1987; Chapter 3). Factors affecting spore germination in soil include dormancy, storage conditions, pH, temperature, moisture, aeration, inorganic ions, growth factors, and presence of other microorganisms (see review by Siqueira et al., 1985).

The innate dormancy periods for spores of VAM fungi vary (Tommerup, 1983) as do periods of viability under cold (4 to 6°C) storage (Ferguson and Woodhead, 1982; Koske, 1981). The mechanisms of observed pH effects on spore germination are unclear. Optimum pH for germination varies with fungal species and may be related to H^+ ion activity, aluminum (Al) tolerance (Siqueira, 1983), or availability of specific nutrients or growth factors (Siqueira et al., 1982; Hepper, 1979). The optimum, minimum, and maximum temperatures for spore germination are variable depending on the physiological adaptation of the fungus to its native environment (Schenck et al., 1975). On the other hand, studies of the effects of moisture on germination indicate that soil water at field

capacity or greater is optimal for spore germination of VAM fungi (Sylvia and Schenck, 1983; Koske, 1981). The upper soil-moisture limit for germination is probably determined by the combined effects of detrimentally high carbon dioxide and low oxygen concentrations. Tolerance limits for CO_2 are variable among VAM fungi (LeTacon et al., 1983). The effects of inorganic ion concentrations on spore germination are variable. In general, N and other salts have little or no effect, while other ions (Mn, Zn, Cu, Ca, and Al), at concentrations commonly found in soil solution, will inhibit spore germination on water agar (Siqueira, 1983; Daniels and Trappe, 1980; Hepper, 1979). The nutritional requirements for spore germination of several species of VAM fungi come from spore reserves. Germination rates have been improved by the addition of growth factors (Siqueira et al., 1982), soil extracts (Daniels and Graham, 1976), root exudates (Graham, 1982), and soil volatile compounds (Hepper, 1978).

Species of Chytridiomycetes and Deuteromycetes have been found as hyperparasites or contaminants of spores of VAM fungi (Siqueira et al., 1984; Sylvia and Schenck, 1983). Bacterial contaminants are also found associated with VAM fungal spores and may affect germination. Mugnier and Mosse (1987) found that spores of G. mosseae which would not germinate spontaneously on water agar did so if contaminated with Streptomyces orientalis. The stimulatory factor appeared to be a volatile substance.

Because of the problem of low VAM fungal colonization reported in Chapter 4, two laboratory Petri-plate experiments were conducted to evaluate the effect of K. pneumoniae on the germination of G.

deserticola spores. This bacterium was chosen on the basis of its potential to enhance plant growth (Chapter 1), VAM fungal spore germination, and root colonization (Chapter 4). Two greenhouse experiments were also performed to evaluate the effect of *K. pneumoniae* on germination of the spores of *G. deserticola* on filter-paper disks buried in sand in which sea oat seedlings were grown. The effect of the bacteria on root colonization by *G. deserticola* and on root growth were also assessed.

Materials and Methods

The *K. pneumoniae* and *G. deserticola* strains are described in Chapters 1 and 3, respectively. The *G. deserticola* was stored in moist sand at 5°C for 16 months before use.

Experiment 1

Two opposing quarters of divided, sterile, polystyrene Petri plates (100 mm x 15 mm) (Fisher Scientific Co.) received 4 mL of 1.5% water agar (Difco Laboratories). The two remaining wells received 4 mL of 1.5% Bacto nutrient agar (Difco Laboratories). Fungal spores were surface disinfested for 2 min in a 1:10 solution of household bleach (5.25% sodium hypochlorite) in sterile water. The spores were then rinsed three times with 20 mL sterile water under a gentle vacuum. Five spores were placed on each plate quarter containing water agar. One-half of the plates received 0.01 mL of nutrient broth (Difco

Laboratories) containing K. pneumoniae (10^6 CFU) in the cells containing nutrient agar. There were nine plates per treatment. All plates were wrapped with Parafilm (American Can Co., Greenwich, CT) and placed in a 28°C growth chamber in the dark. Number of spores germinated (having a germ-tube length of at least 5 μm) by each day (non-cumulative) was determined after 2, 4, 5, 6, 7, 8, and 9 d. Germinated spores were marked and not counted on the following observtion day. After 9 d, the greatest length of hyphal extension from each germinated spore was measured. Standard errors for the means of nine replicates were determined (SAS Institute, Inc., 1985).

Experiment 2

This experiment was set up and analyzed as described for Experiment 1 except that there were 6 plates per treatment, and each water-agar quarter contained ten spores of G. deserticola. Plates were examined after 4, 6, and 8 d.

Experiment 3

Sea oat seedlings were germinated and grown for 10 d in vermiculite, then transplanted (one seedlings per pot) to 77 cm^3 pine-cell tubes (Roy Leach Cone-Tainer Nursery, Canby, OH) containing pasteurized ASRA sand (70°C for 4 h). The VAM fungal spores were surface disinfested as described above and placed in sterile water to a spore density of approximately 5 spores per mL water. The K. pneumoniae culture was prepared for use as inoculum as described in Chapter 1. Gelman polysulfone filter-paper circles (0.2 μm, 25 mm), under gentle

suction in a Millipore filter apparatus, received 1 mL of the spore suspension. One-half of the filters were also inoculated with 1 mL (10^7 CFU) of K. pneumoniae culture. At transplanting, the filters were folded twice and placed approximately 1 cm to the side of roots of sea oats seedlings, at a depth of 3 cm. Plants were fertilized with 10 mL of a 0.1-strength modified Hoagland's solution (Appendix B) containing 0.3 mg P L^{-1} and 10 mg N L^{-1} at the time of planting. The pots were arranged, according to a completely randomized design with 40 replicates of each treatment, in a nonshaded greenhouse. The experiment was conducted between February and May, 1988. The average maximum and minimum temperatures were 27 and 19°C, respectively, and the mean maximum photosynthetic photon flux density was 1433 μmol m^2 s^{-1}.

Randomly-sampled replicates of each treatment were harvested after 2, 4, 6, 28, 48, 68, and 88 d in the pine cells. The filters were removed, any adhering sand was brushed off gently, and stained with 0.05% trypan blue. The cumulative total number of spores and number germinated on each filter were recorded. Root fresh mass, TRL, and percent root colonization were measured after 48, 66, and 88 d. Total root length and percent root length colonized were estimated by methods described in Chapter 4.

Experiment 4

This experiment was set up and analyzed basically as described above for Experiment 3. Randomly-sampled replicates of each treatment were harvested after 5, 8, 28, 66, and 80 d and percent spore germination was determined. Root fresh mass, TRL, and percent root

colonization were measured after 66 and 80 d. The experiment was conducted at the same time and location as Experiment 3.

Results

Experiment 1

Spore germination increased with time up to 9 d (Figure 5-1). Klebsiella pneumoniae in compartments next to those containing VAM fungi spores had no effect on spore germination. Nonetheless, more spores germinated within the first 4 d in the presence of the bacteria than not. The greatest hyphal extension away from the germinated spores, as measured on day 9, was affected by the day on which the spore germinated (Figure 5-2). Spores which germinated on days 4 and 5 in the presence of K. pneumoniae had a significant ($P \leq 0.05$) increase in greatest hyphal extension (as measured on day 9) compared to spores germinated alone.

Experiment 2

Spore germination increased with time, up to 8 d (Figure 5-3). Klebsiella pneumoniae in compartments next to those containing VAM fungi spores had no effect on spore germination. The greatest hyphal extension away from the germinated spores, as measured on day 9, was affected by the day on which the spore germinated (Figure 5-4). Spores which germinated on day 4 in the presence of K. pneumoniae had a significant ($P \leq 0.05$) increase in greatest hyphal extension (as measured on day 9) compared to spores germinated alone.

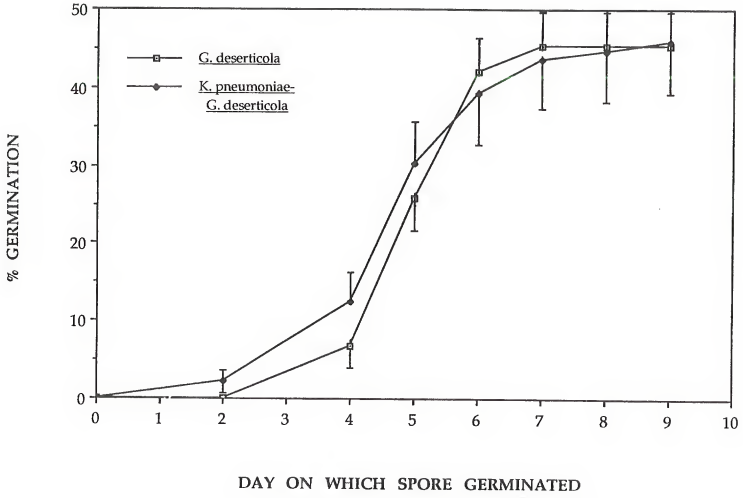

Figure 1. Percentage spore germination of G. deserticola spores, with or without K. pneumoniae, in Experiment 1. Data points represent means of 9 replicates ± S.E.M.

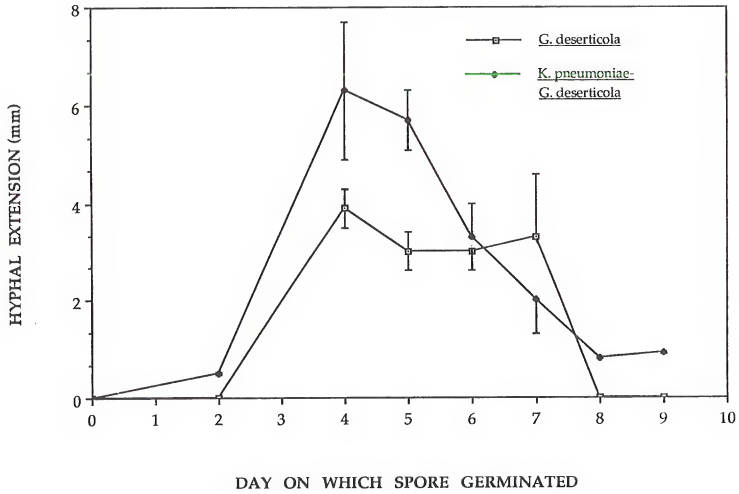

Figure 2. Length of greatest hyphal extension from spores germinated at each day in Experiment 1, ± S.E.M. Number of replicates for G. deserticola are 3,12,and 10 for days 4, 6, and 8, respectively. Number of replicates for K. pneumoniae/G. deserticola are 6, 16 and 13 for the above days.

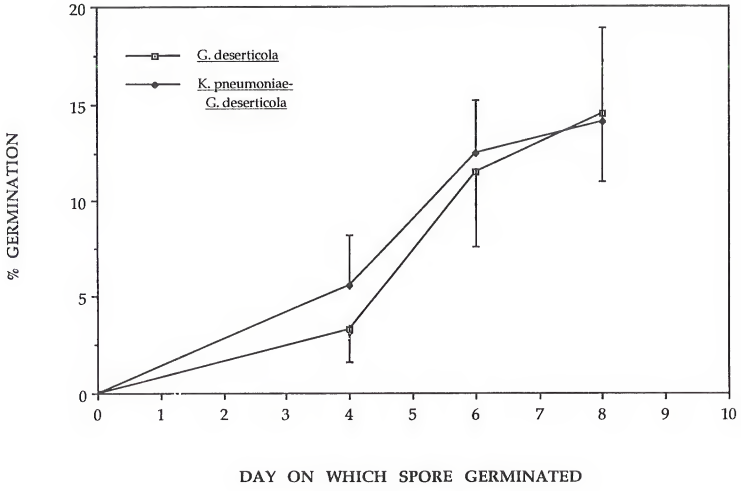

Figure 3. Percentage spore germination of G. deserticola spores, with or without K. pneumoniae, in Experiment 2. Data points represent means of 6 replicates ± S.E.M.

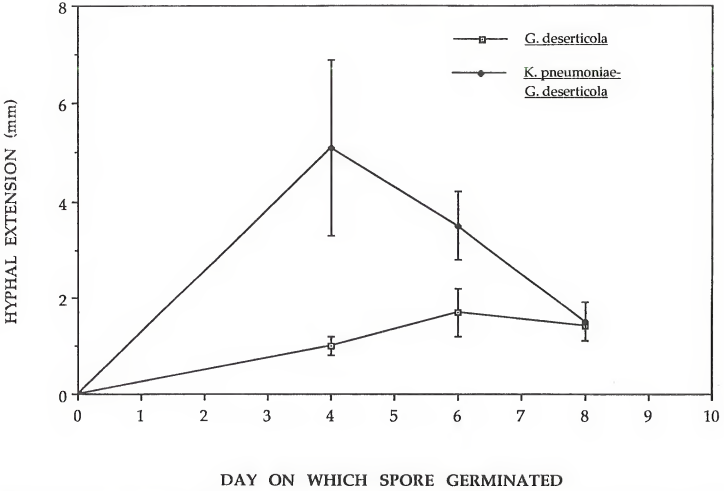

Figure 4. Length of greatest hyphal extension from spores germinated at each day in Experiment 2, ± S.E.M. Number of replicates for G. deserticola are 0, 5, 16, 12, 4, 0, and 0 for days 2, 4, 5, 6, 7, 8, and 9, respectively. Number of replicates for K. pneumoniae/G. deserticola are 2, 8, 15, 8, 4, 1, and 1 for the above days.

Experiment 3

Spore germination increased with time, up to 88 d (Figure 5-5). Percent spore germination at 28 and 48 d was increased by K. pneumoniae, as compared to spores germinating on filter disks without the bacteria. Presence or absence of the bacteria did not effect plant RDM and TRL, or VAM fungal root colonization.

Experiment 4

Spore germination increased with time, up to 80 d (Figure 5-6). Percent spore germination at 66 and 80 d was increased by K. pneumoniae, as compared to spores germinating on filter disks without the bacteria. Presence or absence of the bacteria did not affect plant RDM and TRL, or VAM fungal root colonization.

Discussion

The variability in the results of these exploratory experiments masked a trend toward earlier germination and increased rate of early hyphal growth of VAM fungi incubated in separate compartments of Petri plates with K. pneumoniae than without the bacteria. Significant stimulation of germination occurred on several sampling days when spores were germinated in buried filter disks with K. pneumoniae in the presence of sea oat seedlings. It is possible that this bacteria produces a volatile substance which stimulates the germination process, such as that suggested for Streptomyces orientalis by Mugnier and Mosse (1987), and early hyphal growth. The fact that the stimulation of spore

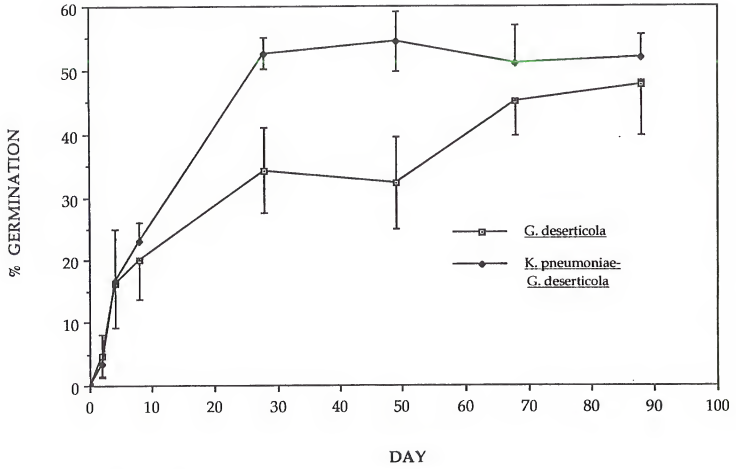

Figure 5. Percentage spore germination of G. deserticola spores, with or without K. pneumoniae, in greenhouse Experiment 1. Data points represent means of 5 replicates ± S.E.M.

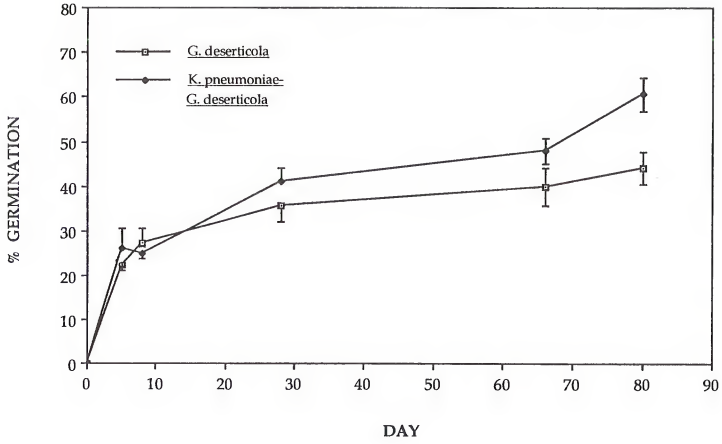

Figure 6. Percentage spore germination of G. deserticola spores, with or without K. pneumoniae, in greenhouse Experiment 2. Data points represent means of 7 replicates ± S.E.M.

germination was more marked in the experiments where bacteria and spores were physically contiguous suggests the involvment of a non-volatile, diffusible substance. Work is needed to identify volatile and non-volatile compounds released by K. pneumoniae and to clarify their effects on germination of G. deserticola spores.

Root colonization by VAM fungi remained low and did not seem to be increased by a bacterially-induced pre-disposition for infection as suggested by Mosse (1962). The low colonization was, however, consistent with the results reported on in Chapter 4 where spores of G. deserticola and G. macrocarpum were used as inoculum. In the present case, germination and early hyphal growth stimulation did not result in earlier or greater fungal colonization of sea oat roots. It is possible that the fungal hyphae grew away from the area in which the bacteria were multiplying, with a resulting decrease in the bacterial influence. If the bacteria were to increase colonization of the root through their effect on the root-cell wall, placing the bacterial inoculum on filter paper at a distance away from the root may have precluded this activity for the length of time involved in this experiment. The bacteria would probably continue to grow and multiply in the filter until the supply of carbohydrates for metabolism (hyphal exudates and dead cellular material, decomposing spores) became insufficient. At that time, growth into the soil and around the root may have occurred.

The slower spore germination in soil as compared to in vitro on water agar may have been the result of suppression by the soil microflora other than K. pneumoniae. Suppression of sporulation in non-sterile soil has been reported for G. macrocarpum (Ross, 1980) and G.

etunicatum (Kitt et al., 1987). Sensitivity to microbial suppression of sporulation differs among VAM species and the mechanism is still being studied.

CHAPTER 6
EFFECT OF BACTERIA AND VAM FUNGI ON GROWTH OF ROOTS OF *UNIOLA PANICULATA* FROM POTTING MIX INTO SAND

Introduction

A major obstacle to survival of commercially-grown, outplanted seedlings of dune grass is initial growth of roots from the potting medium into the dune sand (Augustine et al., 1964). The most important factor in determining successful rooting of transplants is the degree and duration of water stress they experience (Nelms and Spomer, 1983; McKee, 1981; Kratky et al., 1980). While in the greenhouse, the seedlings are given ample nutrients and water to maximize growth before outplanting. The dune environment, on the other hand, is one in which nutrient and water supplies are generally low (Woodhouse, 1982; Willis and Yemm, 1961). The mechanical impedance and reduced porosity of soil material into which the roots are to grow from the potting media also reduces root growth (Nicolosi and Fretz, 1980).

Sylvia found that sea oats colonized with VAM in the nursery and transplanted into replenishment sand in Miami Beach, FL, had increases of 80 and 82% in SDM and TRL over controls in the first growing season (Sylvia, in review). Increased root growth resulting from inoculation with VAM fungi, therefore, may aid in overcoming nutrient and water

stresses encountered by the young transplants. On the other hand, there have been no studies of the effects of bacterial root inoculation on the growth of plants initiated in a commercial potting medium and subsequently transplanted to sand. Greenhouse experiments were therefore established to assess the effects of bacterial root inoculation, alone or in conjunction with VAM fungus inoculation, on the early growth of sea oats initiated in a commercial potting medium and transplanted into ASRA or Miami Beach sand.

Materials and Methods

The K. pneumoniae, A. lipoferum, and A. denitrificans isolates were the same as described in Chapter 1. The G. deserticola-G. macrocarpum spore inoculum was prepared as described in Chapter 3.

Greenhouse Culture Method

Experiment 1. Sea oat seeds were germinated in vermiculite for 14 d, then transplanted into the 23 cm^3 cells of TODD Planter Flats (Speedling, Inc., Sun City, FL) containing Metro Mix 200 growing medium (Grace Horticultural Products, Cambridge, MA). Immediately prior to transplanting, 1 mL of a suspension containing K. pneumoniae or A. denitrificans (10^7 CFU mL^{-1}) or sterile water (control) was placed in each transplanting hole. The cultures were prepared for use as inoculum as described in Chapter 1. Plants were fertilized with 10 mL of a 0.1-strength modified Hoagland's solution (Appendix B) containing 0.3 mg P L^{-1} and 10 mg N L^{-1} at the time of planting and every 7 d thereafter.

After 28-d growth, seedlings were transplanted from the potting mix to 620-mL Deepot inserts containing pasteurized ASRA sand (70°C for 4 h). Selected chemical characteristics of the ASRA sand are given in Appendix A. Plants were fertilized with 10 mL of a 0.1-strength modified Hoagland's solution containing 0.3 mg P L^{-1} and 10 mg N L^{-1} at the time of planting and every 14 d thereafter. The Deepot inserts were arranged in a completely randomized design. The experiment was conducted between April and July, 1987, in a nonshaded greenhouse. The average maximum and minimum temperatures were 30 and 23°C, respectively, and the average maximum photosynthetic photon flux density was 1804 μmol m^2 s^{-1}.

Experiment 2. This experiment was set up as described above. Ten days prior to planting in the potting mix, approximately 50 spores of the VAM fungi in 2-mL sterile water were placed at a depth of about 4 cm in the appropriate Speedling tray cells. Control cells received 2 mL of water used to wash the spores on the 45-μm screen from which the spores were collected. Roots were inoculated with K. pneumoniae, A. lipoferum, or A. denitrificans at the time of planting as described above. Seedlings were transplanted to ASRA sand after 35 d, and fertilized as in Experiment 1. The Deepot inserts were arranged in a completely randomized design. The experiment was conducted between June and September, 1987, in a nonshaded greenhouse. The average maximum and minimum temperatures were 31 and 23°C, respectively, and the average maximum photosynthetic photon flux density was 1765 μmol m^2 s^{-1}.

Sampling and Analysis

For experiment 1, 10 randomly-selected replicates of each treatment were harvested after 28 d in the Metro Mix (at the time of transplanting to sand), after 28-d growth in sand, and after 62-d growth in sand, and analyzed for RDM, TRL, HTT, SDM, shoot N, and shoot P as described in Chapter 1. Roots growing in the sand were clipped at the sand-potting mix interface and analyzed separately from those in the Metro Mix. For Experiment 2, 15 randomly-selected replicates of each treatment were harvested after 35 d in the Metro Mix (at the time of transplanting to sand), and after 56-d growth in sand and analyzed as described in Chapter 1. The root mass was prepared for total root length and root length colonized by VAM fungi as described in Chapter 3. The data were analyzed as described in Chapter 2. Percentage data were subjected to the arcsine transformation for analysis.

Results

Experiment 1

There were no differences in RDM, TRL, HTT, SDM, percent N, total N, or total P after 28-d growth in Metro Mix (Tables 6-1 and 6-2). Control plants had greater percent shoot P than did plants inoculated with either K. pneumoniae or A. denitrificans. Twenty eight days after transplanting to the ASRA sand, the plants inoculated with K. pneumoniae had lower RDM in Metro Mix (along with A. denitrificans), RDM in sand, TRL in sand, and HTT, SDM, and total N in both substrates, than did control plants (Tables 6-1 to 6-4). Klebsiella pneumoniae and A.

Table 6-1. F values from ANOVA for Experiment 1.

	---Metro Mix---		-----Sand-----			
	Root dry mass (mg)	Total root length (cm)	Root dry mass (mg)	Total root length (cm)	Height tallest tiller (cm)	Shoot dry mass (mg)
	----------28 d in Metro Mix----------					
Microbial Inoculum	.4170	.2848	----	----	.3792	.5357
C.V. (%)	39	34	----	----	22	37
Error d.f.	27	27	----	----	27	27
	----------28 d in Metro Mix plus 28 d in sand------					
Microbial Inoculum	.0001	.8682	.0001	.0001	.0001	.0001
C.V. (%)	31	42	51	61	29	26
Error d.f.	27	27	27	27	27	27
	----------28 d in Metro Mix plus 62 d in sand------					
Microbial Inoculum	.0541	.0044	.0012	.4638	.0280	.0004
C.V. (%)	35	42	37	46	17	27
Error d.f.	27	27	27	27	27	27

Table 6-2. F values from ANOVA for Experiment 1.

	Shoot N (%)	Total shoot N	Shoot P (%)	Total shoot P
	----------28 d in Metro Mix----------			
Microbial Inoculum	.3623	.2084	.0119	.4366
C.V. (%)	15	19	8	25
Error d.f.	6	6	9	9
	------28 d in Metro Mix plus 28 d in sand------			
Microbial Inoculum	.5285	.0133	.0001	.0001
C.V. (%)	21	15	15	16
Error d.f.	6	6	12	12
	------28 d in Metro Mix plus 62 d in sand------			
Microbial Inoculum	.0786	.1694	.0001	.0146
C.V. (%)	22	20	10	27
Error d.f.	6	6	12	12

Table 6-3. Main effects of bacterial root inoculation on growth of sea oats.

Inoculation treatment	----Metro Mix---- Root dry mass (mg)	Total root length (cm)	------Sand------ Root dry mass (mg)	Total root length (cm)	Height tallest tiller (cm)	Shoot dry mass (mg)
	----------28 d in Metro Mix plus 28 d in sand----------					
Control	35.9 A[a]	91 A	60.6 A	331 A	31 A	210.0 A
K. pneumoniae	17.5 B	53 A	5.2 B	29 B	13.2 B	94.1 B
A. denitrificans	25.2 B	89 A	48.8 A	311 A	30 A	189.9 A
	----------28 d in Metro Mix plus 62 d in sand----------					
Control	27.1 A	111 A	117.3 A	589 A	43 A	332.0 A
K. pneumoniae	25.0 A	68 B	63.1 B	536 A	35 B	212.9 B
A. denitrificans	36.2 B	141 B	136.3 A	693 A	44 A	392.8 A

[a] Each value represents the mean of ten replicates. Microbial treatment means with the same letter as the Control mean, within sample date, are not significantly different from the Control mean ($P \leq 0.05$).

Table 6-4. Main effects of bacterial root inoculation on growth of sea oats.

Inoculation Treatment	Total shoot N (mg)	Shoot P (%)	Total shoot P (mg)
	-------28 d in Metro Mix plus 28 d in sand-------		
Control	1.34 A[a]	0.02 A	0.05 A
K. pneumoniae	0.81 B	0.02 A	0.02 B
A. denitrificans	1.37 A	0.01 B	0.03 B
	-------28 d in Metro Mix plus 62 d in sand-------		
Control	1.95 A	0.09 A	0.03 A
K. pneumoniae	1.70 A	0.08 A	0.03 A
A. denitrificans	2.40 A	0.15 B	0.05 B

[a] Each value for N represents the mean of three replicates, while those for P represent the mean of five replicates. Microbial treatment means with the same letter as the Control mean, within sample date, are not significantly different from the Control mean ($P \leq 0.05$).

denitrificans caused decreased total P, and A. denitrificans decreased percent P, compared to control plants. After 62-d growth in sand, plants inoculated with K. pneumoniae had lower TRL (Metro Mix), RDM (sand), HTT, and SDM compared to controls. Inoculation with A. denitrificans resulted in increased RDM (Metro Mix), and percent and total shoot P compared to controls.

Experiment 2

After 35 d in Metro Mix, plants inoculated with K. pneumoniae or A. denitrificans had decreases in HTT and SDM, as compared to controls (Tables 6-5 and 6-6). Plants inoculated with A. lipoferum, alone or in combination with the fungi, had increased RDM as compared to controls, while TRL was increased by this bacteria/fungi combination only. Roots of plants inoculated with the combination of VAM fungi and K. pneumoniae had higher percent-root colonization than those inoculated with the fungus in combination with A. lipoferum or A. denitrificans. There was no fungal colonization in plants inoculated with the fungus alone. Fifty-six days after transplanting into sand, the only significant increase related to microbial inoculation was in HTT of plants inoculated with the VAM fungi/A. lipoferum combination. Microbial inoculation resulted in decreases in all growth and nutrient parameters as shown in Tables 6-5, 6-7, and 6-8. Root colonization by the VAM fungi did not appear to spread from the initial point of inoculation in plants inoculated with the fungi and A. lipoferum or A. denitrificans. In any case, the sampling for colonization determination was not intensive enough to pick the colonization up in roots of plants dually

Table 6-5. F values from ANOVA for Experiment 2.

	--Metro Mix--		----Sand----			
	Root dry mass (mg)	Total root length (cm)	Root dry mass (mg)	Total root length (cm)	Height tallest tiller (cm)	Shoot dry mass (mg)
	----------	-----35 d in Metro Mix----------				
Microbial Inoculum	.0175	.0009	----	----	.0172	.0002
C.V. (%)	68	59	----	----	25	41
Error d.f.	82	82	----	----	83	83
	-------35 d in Metro Mix plus 56 d in sand-------					
Microbial Inoculum	----	----	.0126	.0002	.0001	.0059
C.V. (%)	----	----	41	29	10	22
Error d.f.	----	----	71	71	71	71

Table 6-6. Effect of root inoculation with bacteria and a mixture of G. deserticola and G. macrocarpum spores (VAM) on growth of sea oat seedlings in Metro Mix 200 for 35 d.

Inoculation Treatment	Root dry mass (mg)	Root colonized (%)	Total root length (cm)	Height tallest tiller (cm)	Shoot dry mass (mg)
Control	4.3 A[a]	----	30 A	14 A	29.8 A
VAM	5.0 A	0 A	46 A	15 A	29.9 A
K. pneumoniae	3.0 A	----	25 A	11 B	12.9 B
A. lipoferum	7.0 B	----	36 A	14 A	25.2 A
A. denitrificans	3.5 A	----	22 A	10 B	14.1 B
VAM/K. pneumoniae	4.6 A	50 B	30 A	13 A	30.9 A
VAM/A. lipoferum	7.3 B	4 A	59 B	14 A	33.7 A
VAM/A. denitrificans	3.3 A	12 A	33 A	13 A	23.0 A

[a] Each value represents the mean of at least 10 replicates. Means with the same letter are not significantly different ($P \leq 0.05$).

Table 6-7. F values from ANOVA for Experiment 2.

	Root colonized (%)	Shoot N (%)	Total shoot N	Shoot P (%)	Total shoot P
	----------35 d in Metro Mix----------				
Microbial Inoculum	.0001	----	----	----	----
C.V. (%)	91	----	----	----	----
Error d.f.	37	----	----	----	----
	-------35 d in Metro Mix plus 56 d in sand------				
Microbial Inoculum	.7624	.0198	.0102	.0001	.0224
C.V. (%)	42	19	24	24	33
Error d.f.	20	16	16	32	32

Table 6-8. Effect of root inoculation with bacteria and a mixture of G. deserticola and G. macrocarpum (VAM) on growth of sea oat seedlings in ASRA sand for 56 d, after prior growth in Metro Mix for 35 d.

Inoculation treatment	Root dry mass (mg)	Total root length (cm)	Height tallest tiller (cm)	Shoot dry mass (mg)	Shoot N (%)	Total shoot N (mg)	Shoot P (%)	Total shoot P (mg)
Control	204.2 A[a]	1347 A	40 A	309.4 A	0.89 A	2.98 A	0.18 A	0.49 A
VAM	175.9 A	967 B	36 B	275.5 A	0.79 A	2.67 A	0.10 B	0.27 B
K. pneumoniae	173.6 A	886 B	38 A	256.2 A	0.43 B	1.01 B	0.16 A	0.43 A
A. lipoferum	108.3 B	729 B	35 B	232.0 B	1.05 A	2.39 A	0.12 B	0.27 B
A. denitrificans	142.3 A	946 B	40 A	300.0 A	0.93 A	2.61 A	0.10 B	0.34 A
VAM/ K. pneumoniae	193.8 A	1149 A	42 A	304.2 A	0.69 A	1.21 B	0.18 A	0.50 A
VAM/ A. lipoferum	219.3 A	1405 A	43 B	329.5 A	1.03 A	3.70 A	0.10 B	0.32 B
VAM/ A. denitrificans	139.1 B	1107 A	37 A	255.4 B	0.86 A	2.11 A	0.10 B	0.35 A

[a] Each value for shoot and root growth represents the mean of at least eight replicates, while values for shoot N represent three replicates and values for P represent the mean of five replicates. Microbial treatment means with the same letter as the Control mean are not significantly different from the Control mean ($P \leq 0.05$).

inoculated with these microbes. Colonization of roots by the fungi was the same whether plants received K. pneumoniae as co-inoculant or the fungi alone (32 and 30%,respectively).

Discussion

Bacterial root inoculation of sea oats growing in potting medium and transplanted into sand generally had either no effect on plant growth or a negative one. The reason for the negative effect of inoculation with K. pneumoniae on sea oat root growth in Metro Mix compared to sand, as seen in previous experiments (Chapter 2), is not known. It is possible that the organic C component of the Metro Mix provided a substrate for the bacteria, allowing cell proliferation and competition with the plants for available nutrients (Tinker, 1984). On the other hand, the bacteria may have produced phytotoxic metabolic by-products (eg. phenolic and short-chain fatty acids, ethylene) in sufficient quantity to inhibit root growth (Marschner, 1986).

Initially, K. pneumoniae stimulated VAM fungal root colonization, whereas the VAM fungi alone were slow to colonize the roots of seedlings growing in the potting medium. The low colonization of roots by the VAM fungi was consistent with the results of Biermann and Linderman (1983) who found peat-, perlite-, or vermiculite-based growing media to inhibit VAM fungal root colonization. Additions of clay or silty loam soil alleviated this problem. The authors suggested that the beneficial effects of these materials may be to alter the matric potential of, or P diffusion rate in, the growing medium.

Increases in TRL and RDM resulting from inoculation of sea oat seedlings outplanted to Miami Beach (Sylvia, in review) were not seen here. Although it is likely that fungal hyphae growing into the sand aided in water and nutrient uptake, the plants were not under water or nutrient stress in the present set of experiments. This may account for the general lack of significant increases in growth due to VAM fungus inoculation.

CHAPTER 7
SUMMARY AND CONCLUSIONS

Inoculation of roots of sand-dune plants with bacteria or VAM fungi has been found to result in enhanced plant growth and nutrient uptake (Sylvia and Burks, 1988; Abdel Wahab and Wareing, 1980). A series of experiments were conducted to determine whether inoculation of sea oats with bacteria, including indigenous sea oat rhizosphere bacteria and known N_2-fixers, alone or in combination with the VAM fungi G. deserticola and G. macrocarpum, would enhance sea oat growth in sand under greenhouse conditions. A striking result was the inability of known N_2-fixing bacteria (K. pneumoniae and two Azospirillum spp.) to provide the plants with fixed N. Specific relations between strains of N_2-fixing bacteria and corn hybrids was reported by Saric et al. (1987). In their work, the effect of strains of Azosprillum and Klebsiella on N content of corn ranged from highly positive to highly negative. In the case of the ASRA sand, which contains about 0.5 mg L^{-1} inorganic N and to which no fertilizer N was added, the bacteria appeared to compete with the plants for available N. Insufficient carbohydrate probably limited N_2 fixation even when N was adequate. On the basis of the first set of experiments (Chapter 1), there was no statistically strong support for the hypothesis that bacterial root inoculation would enhance sea oat growth and nutrient uptake. However, the hypothesis was not rejected outright as there were indications in the results presented in Chapter 2

that root inoculation with K. pneumoniae, would have a positive effect on root and shoot growth under certain conditions and variability in growth of this native grass was high. It was concluded that these effects were not the result of increased plant-available N from bacterial N_2 fixation. Research to match sea oats with appropriate N_2-fixing organisms is needed.

In the second set of experiments, which looked at the effect of P availability and plant-growth responses, it was found that, overall, bacterial inoculation of sea oats did not increase the plant uptake of water-soluble P over controls. However, there were nonsignificant increases in plant growth and shoot P associated with bacterial inoculation when adequate P was added for plant growth, suggesting that its uptake may have been mediated by the rhizosphere bacterial population. When a sparingly-soluble P source was added to the ASRA sand, inoculation with K. pneumoniae resulted in increased growth and shoot P. Nonsignificant increases in plant growth associated with inoculation with the bacterial isolates was seen in plants grown in Miami Beach sand amended with sparingly-soluble calcium phosphate. The fact that shoot-P uptake was not affected by inoculation indicates that probably a mechanism other than increased P availability was responsible although it is possible that P was being sequestered in the roots.

When sea oats growing in ASRA sand were dually inoculated with either K. pneumoniae or A. denitrificans, and the combined G. deserticola-G. macrocarpum VAM fungus inoculum there was no consistent evidence of a synergistic effect between the bacteria and fungi on plant growth. Nonsignificant positive effects of colonization of roots by VAM

fungi on plant-P uptake were seen. In the Miami Beach sand, all inocula increased growth (nonsignificantly) with a synergistic effect of VAM fungi and the bacteria K. pneumoniae-A. lipoferum evident when soluble P was added. The presence of the bacterial inoculum had no effect on colonization of roots by VAM fungi in sand when spore inoculum was used. The lack of effect indicates that the bacteria did not enhance spore germination in this growing medium. The fact that colonization was enhanced when a spore-hyphae-root inoculum was used in conjunction with bacterial inoculation suggests the bacteria may have affected the plant-root-cell wall, thereby increasing the susceptibility of the plant tissue to fungal attack. We did not reject the hypothesis that dual inoculation of sea oats with a VAM fungus and a bacterial isolate would enhance plant growth and nutrient content. However, further study is required to understand interactions of the bacteria and fungi in the rhizosphere in order to choose appropriate co-inoculants and minimize experimental variability.

Further insights into the interaction between bacterial and fungal co-inoculants were achieved by determining the effects of K. pneumoniae on VAM fungus spore germination and early hyphal growth. Although the results were not always statistically significant, the hypothesis that K. pneumoniae produced a volatile substance which affected spore germination and hyphal growth was not rejected as there was a trend toward bacterially-induced early spore germination and faster hyphal growth. These results warrant further research as they are consistent with the work of Mugnier and Mosse (1987), yet the results of dual inoculation experiments reported on here indicate that such effects do

not necessarily result in earlier or more successful VAM fungal root colonization. The fungal hyphae growing out of the filter disk may leave the area in which the bacteria are capable of influencing their growth. Bacteria may have been slow to proliferate in the low-carbon sand and placement of the bacterial inoculum at a distance from the root may have reduced the chances of a bacterial effect on root-cell-wall integrity and, consequently, on the susceptibility of the root to VAM fungal infection.

The results of experiments in Chapter 6 do not lead to acceptance of the hypothesis that inoculation of sea oat seedlings with bacteria and VAM fungi while growing in a commercial potting medium would enhance the plants' ability to adapt to the sand-dune environment upon outplanting. Glomus deserticola and G. macrocarpum have been shown to enhance the growth of outplanted sea oats (Sylvia, in review). The negative effect of K. pneumoniae on growth of sea oat seedlings in the Metro Mix raises interesting questions. Further research into the possible role of bacterially-produced phytohormones or stimulation of phytopathogenic rhizosphere microorganisms is warranted. Klebsiella pneumoniae stimulated VAM fungal root colonization in Metro Mix, whereas the VAM fungi alone were slow to colonize the roots of seedlings growing in this growing medium. Biermann and Linderman (1983) suggested that the beneficial effects of adding soil may be to alter the matric potential of, or P diffusion rate in, the growing medium. It is possible that the presence of the bacteria affected these same parameters although other factors, such as bacterial action on root-cell walls as mentioned above, may also be acting to achieve an increased VAM

fungus root colonization.

The results of these experiments indicate the need for further evaluation of the ability of bacterial and fungal inoculation of pioneer sand dune grasses to enhance plant growth and dune stabilization. Careful consideration must be given to matching bacterial and fungal co-inoculants with each other, with the growing medium, and with the fertilizer regime in order to achieve a successful and economical system.

APPENDIX A
CHEMICAL AND PHYSICAL CHARACTERISTICS
OF THE ANASTASIA STATE RECREATION AREA AND MIAMI BEACH SANDS

	Anastasia State Recreation Area	Miami Beach
Water slurry pH (1:1)	9.1	9.6
Electrical conductivity	74.7	42.0
NH_4-N	0.10	0.10
NO_3-N	0.37	0.00
Ca	5906.7	6210.0
Water-extractable P	0.075	0.011
Mehlich-I extractable P	633.3	3.3
% soil particles:		
$\leq$ 0.05 mm	0.0	0.6
>0.05 - <0.5 mm	3.4	59.2
>0.05 - < 2 mm	96.6	40.2

[a] All units are mg L^{-1}, except electrical conductivity (dS m^{-2}).

APPENDIX B
MODIFIED HOAGLAND'S SOLUTION USED IN GREENHOUSE EXPERIMENTS

Nutrient	Source	Concentration ($mg\ L^{-1}$)
NH_4-N	NH_4NO_3	10.0
NO_3-N	NH_4NO_3	10.0
K	KCl, KH_2PO_4	23.5
P	KH_2PO_4	3.1
Ca	$CaCl \cdot 2H_2O$	20.0
Mg	$MgSO_4 \cdot 7H_2O$	4.8
S	$MgSO_4 \cdot 7H_2O$	6.4
Fe	0.1 M Fe-EDTA	5.6
Micronutrients	Hoagland's Solution A (Hoagland and Arnon, 1950)	

APPENDIX C
RANGES OF MEANS OF GROWTH AND SHOOT NUTRIENT VALUES FOR SEA OATS INOCULATED WITH BACTERIA IN ANASTASIA STATE RECREATION AREA SAND, WITH OR WITHOUT N ADDITIONS

	Inoculated	Control
0 mg N L1		
RDM (mg)	30.9 (Ab)[a] - 42.6 (Kp)	30.5
TRL (cm)	254 (Al) - 355 (Bp)	229.0
HTT (cm)	15 (T) - 17 (Ab)	16.6
SDM (mg)	20.4 (T) - 40.6 (Kp)	30.9
% shoot N	0.30 (T) - 0.84 (Bp)	1.71
Total shoot N (mg)	0.08 (T) - 0.57 (Ad)	0.53
% shoot P	0.19 (T and Bp) - 0.25 (Kp and Ad)	0.23
Total shoot P (mg)	0.04 (Al) - 0.11 (Kp and Ad)	0.07
2 mg N L1		
RDM (mg)	112.4 (Bp) - 160.8 (Kp)	106.9
TRL (cm)	539 (T) - 737 (Kp)	534.5
HTT (cm)	29 (Ad) - 32 (Kp)	29.7
SDM (mg)	66.1 (Bp) - 152.9 (Kp)	85.8
% shoot N	0.82 (Al) - 1.63 (T)	2.21
Total shoot N (mg)	0.72 (Ab) - 1.42 (Kp)	2.32
% shoot P	0.09 (Kp) - 0.14 (Ad)	0.11
Total shoot P (mg)	0.09 (Ab) - 0.16 (Bp)	0.12

[a] Microbial inocula are designated as follows: Kp = *K. pneumoniae*, Al = *A. lipoferum*, Ab = *A. brasiliense*, Bp = *B. polymyxa*, Ad = *A. denitrificans*, T = T3E3+T3E10

APPENDIX D
MEANS OF GROWTH AND SHOOT NUTRIENT VALUES FOR SEA OATS INOCULATED WITH BACTERIA IN ANASTASIA STATE RECREATION AREA SAND, WITH OR WITHOUT N ADDITIONS

	Root dry mass (mg)	Total root length (cm)	Height tallest tiller (cm)	Shoot dry mass (mg)	Shoot N (%)	Total N (mg)	Shoot P (%)	Total P (mg)
0 mg N L^{-1}								
Control	15.1	272	16	33.5	1.62	0.55	0.13	0.45
K. pneumoniae	20.1	377	16	36.2	1.55	0.50	0.18	0.70
A. denitrificans	20.4	402	16	34.4	1.88	0.67	0.15	0.56
2 mg N L^{-1}								
Control	35.4	493	32	86.1	3.43	3.00	0.10	0.08
K. pneumoniae	51.8	786	34	111.6	3.04	3.39	0.10	0.13
A. denitrificans	42.5	543	27	91.5	3.14	3.25	0.13	0.14

APPENDIX E
MEAN VALUES OF GROWTH DATA FOR SEA OATS INOCULATED WITH BACTERIA IN ANASTASIA STATE RECREATION AREA SAND, WITH OR WITHOUT SOLUBLE P ADDITIONS

	Root dry mass (mg)	Total root length (cm)	Height tallest tiller (cm)	Shoot dry mass (mg)	Shoot N (%)	Total N (mg)	Shoot P (%)	Total P (mg)
0 mg P L^{-1}								
Control	16.9	209	23	86.4	2.10	1.81	0.05	0.04
K. pneumoniae	12.9	174	18	64.8	2.63	1.96	0.05	0.04
A. lipoferum	19.5	174	21	67.3	2.80	1.59	0.06	0.05
A. denitrificans	17.2	224	23	78.4	2.94	2.16	0.05	0.04
2 mg L^{-1} P								
Control	11.0	100	18	60.7	3.49	3.04	0.06	0.04
K. pneumoniae	21.5	222	28	108.6	2.77	1.98	0.08	0.08
A. lipoferum	34.5	303	30	131.2	2.15	1.91	0.07	0.09
A. denitrificans	15.8	182	25	78.3	2.96	2.09	0.07	0.05

APPENDIX F
MEAN VALUES OF SHOOT NUTRIENT DATA FOR SEA OATS INOCULATED WITH BACTERIA IN ANASTASIA STATE RECREATION AREA SAND, WITH OR WITHOUT $CaHPO_4$

	Shoot N (%)	Total N (mg)	Shoot P (%)	Total P (mg)
Control	1.31	1.75	0.16	0.20
K. pneumoniae	1.26	2.04	0.18	0.27
A. lipoferum	1.34	1.82	0.17	0.22
A. denitrificans	1.35	2.01	0.18	0.25

APPENDIX G
MEAN VALUES OF GROWTH DATA FOR SEA OATS INOCULATED WITH BACTERIA IN MIAMI BEACH SAND, WITH OR WITHOUT $CaHPO_4$

	Root dry mass (mg)	Total root mass (cm)	Height tallest tiller (cm)	Shoot dry mass (mg)	Shoot N (%)	Total N (mg)	Shoot P (%)	Total P (mg)
Control	51.8	506	33	222.8	1.01	2.27	0.18	0.36
K. pneumoniae	92.0	629	39	290.3	0.69	2.19	0.13	0.36
A. lipoferum	79.1	704	40	257.0	0.92	2.48	0.12	0.29
A. denitrificans	112.4	775	41	265.6	0.72	1.99	0.11	0.30

APPENDIX H
MEAN VALUES OF SHOOT NUTRIENT DATA FOR SEA OATS INOCULATED WITH BACTERIA AND VAM FUNGI SPORES IN ANASTASIA STATE RECREATION AREA SAND

	Root dry mass (mg)	Root colonized (%)	Total root length (cm)	Height tallest tiller (cm)	Shoot dry mass (mg)
Control	39.8	----	543	28	165.0
VAM fungi	42.9	12	549	31	176.3
K. pneumoniae	46.0	----	984	21	140.0
K. pneumoniae/ VAM fungi	44.6	8	637	29	152.7

	Shoot N (%)	Total shoot N (mg)	Shoot P (%)	Total shoot P (mg)
Control	2.57	7.04	0.07	0.11
VAM fungi	3.16	7.68	0.08	0.16
K. pneumoniae	2.76	7.96	0.06	0.10
K. pneumoniae/ VAM fungi	2.58	6.75	0.07	0.11

APPENDIX I
MEAN VALUES OF SHOOT NUTRIENT DATA FOR SEA OATS INOCULATED WITH BACTERIA AND VAM FUNGI SPORES IN ASRA SAND

	Root dry mass (mg)	Root colonized (%)	Total root length (cm)	Height tallest tiller (cm)	Shoot dry mass (mg)
Control	24.8	----	281	28	106.7
VAM fungi	16.8	19	187	27	90.1
A. denitrificans	18.7	----	211	24	85.5
A. denitrificans/ VAM fungi	44.6	23	268	24	83.2

	Shoot N (%)	Total shoot N (mg)	Shoot P (%)	Total shoot P (mg)
Control	2.55	4.02	0.07	0.08
VAM fungi	3.07	4.10	0.10	0.09
A. denitrificans	2.48	4.26	0.07	0.07
A. denitrificans/ VAM fungi	2.43	4.31	0.11	0.10

APPENDIX J
MEAN VALUES OF GROWTH AND SHOOT NUTRIENT DATA FOR SEA OATS INOCULATED WITH BACTERIA AND VAM FUNGI ROOT-HYPHAE-SPORE INOCULUM IN MIAMI BEACH SAND

	Root dry mass (mg)	Total root length (cm)	Height tallest tiller (cm)	Shoot dry mass (mg)	Shoot P (%)	Total shoot P (mg)
	----------0 mg P L^{-1}----------					
Control	5.3	94	13	37.8	0.09	0.11
VAM fungi	29.1	189	26	82.6	0.11	0.12
K. pneumoniae/ *A. lipoferum*	25.0	244	22	104.3	0.17	0.36
VAM fungi/ *K. pneumoniae*/ *A. lipoferum*	13.9	136	20	60.3	0.11	0.08
	----------2 mg P L^{-1}----------					
Control	15.1	127	17	55.3	0.08	0.06
VAM fungi	32.7	219	26	95.1	0.12	0.14
K. pneumoniae/ *A. lipoferum*	34.5	295	22	94.1	0.15	0.27
VAM fungi/ *K. pneumoniae*/ *A. lipoferum*	55.5	344	35	141.7	0.14	0.22

APPENDIX K

MEAN VALUES OF GROWTH AND SHOOT NUTRIENT DATA FOR SEA OATS INOCULATED WITH BACTERIA AND VAM FUNGI ROOT-HYPHAE-SPORE INOCULUM IN ASRA SAND

Inoculation treatment	Root dry mass (mg)	Root colonized (%)	Total root length (cm)	Height tallest tiller (cm)	Shoot dry mass (mg)	Shoot N (%)	Total shoot N (mg)	Shoot P (%)	Total shoot P (mg)
	----------0 mg P L^{-1}----------								
Control	50.4	----	425	30	151.4	1.42	1.01	0.10	0.12
VAM	65.2	36	420	35	150.8	0.99	1.50	0.10	0.14
K. pneumoniae/ *A. lipoferum*	72.7	----	467	34	178.2	1.03	1.50	0.08	0.11
VAM/*K. pneumoniae*/ *A. lipoferum*	39.2	34	291	31	141.0	1.07	2.07	0.13	0.15
	----------0.05 mg P L^{-1}----------								
Control	40.0	----	354	28	130.9	0.83	1.36	0.06	0.10
VAM	62.8	41	386	33	142.8	0.98	1.39	0.10	0.14
K. pneumoniae/ *A. lipoferum*	58.2	----	398	31	142.9	1.04	1.93	0.11	0.18
VAM/*K. pneumoniae*/ *A. lipoferum*	35.0	43	289	29	131.6	1.23	2.26	0.13	0.18

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